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6381

Effect of an Injury Confined to One Rat Incisor and its Investing Tissues Upon the Other Incisors.

I. SCHOUR. (Introduced by W. F. Petersen.)

From the Department of Dental Histology, University of Illinois, and the Department of Anatomy, University of Chicago.

The effect of experimental injuries to the lower incisors and their investing tissues was studied in 22 rats of 21-450 days of age and over a period of from 31 to 158 days. The experimental animals were selected at random from the normal rats of 4 different colonies and over a period of more than 2 years in order to avoid the possibility of using a susceptible strain. Litter-mate controls of 17 experimental animals were studied in addition to 63 normal animals obtained from similar sources.

The rate of eruption was measured and weekly records of the gross appearance of the teeth and jaws were kept throughout the duration of the experiments. A radiographic, anatomic and histologic study was made of the teeth after death. The experimental injuries were in the nature of fracturing of one or 2 lower incisors and their investing tissues. In some cases, one lower incisor and its investing tissues were fractured while the enamel epithelium of the other lower incisor was injured with a fine needle. In no case were the upper incisors injured.

The control animals showed a normal rate of eruption and a normal radiographic and histologic picture of the dental tissues. Those of the experimental animals that were studied preceding the injuries showed a normal rate of eruption and a normal appearance of the teeth.

In 85% of the experimental animals, the result of the injuries were: (a) Considerable retardation in eruption and (b) more or less severe histo-pathologic changes in the dental tissues, particularly the enamel and the enamel epithelium, in the injured teeth and in varying degrees in the uninjured teeth as well.

The changes were progressive and more or less permanent. In some cases they appeared within a week following the operations.

Series of histologic and experimental studies are being made to investigate the possible mechanical (functional), neural, cytolytic, or other explanations of this reaction, which reminds one of "sympathetic" ophthalmia. The first explanation seems to be ruled out in certain cases where practically normal function was maintained throughout the post-operative period.

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Relation of Induced Anoxemia to the Pain of Muscular Exercise.*

MILTON KISSIN. (Introduced by L. N. Katz.)

From the Heart Station, Michael Reese Hospital, Chicago.

Exercise of the forearm during the time that the arterial blood oxygen is progressively lowered will initiate pain in the exercised muscles. The pain disappears when the blood is suddenly saturated with oxygen although the exercise be continued. If the same exercise is performed at the same rate while the arterial blood remains saturated with oxygen, no pain appears. Increasing the frequency of the exercise may cause pain to appear even when the blood is saturated with oxygen. Decreasing the frequency of exercise while maintaining the arterial oxygen at a low level may result in no pain.

This work can be correlated with the reports of MacWilliam and Webster[1] and of Lewis, Pickering, and Rothschild,[2] who found that

* Aided by a grant from the Herbert L. Celler Fellowship Foundation, New York, and the Frederick K. Babson Fund of the Michael Reese Hospital, Chicago.

[1] MacWilliam, J. A., and Webster, W. J., *Br. Med. J.*, 1923, **1**, 51.

[2] Lewis, T., Pickering, G. W., and Rothschild, P., *Heart*, 1931, **15**, 359.

exercise of a muscle that had been made ischemic, results in pain. Their work showed that inadequate blood supply to a working muscle causes pain. In my experiments the blood supply, save for the lowered oxygen content, was unaltered in composition, yet pain appeared during exercise. This shows that oxygen want is the the chief contributing cause of pain developing in an exercised muscle. It is, therefore, suggested that the cause of pain in muscular exercise is the incomplete oxidation of the products of muscle metabolism. The same process may be responsible for the pain of angina pectoris (Rothschild and Kissin[3]) and of intermittent claudication.

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Filter-Passing Bacteria in Polluted Water.

H. E. MC DANIELS AND JOHN NEAL. (Introduced by L. Arnold.)

From the Research Laboratories, State Department of Public Health, and Department of Bacteriology and Preventive Medicine, University of Illinois College of Medicine.

References to epidemics of gastro-enteritis attributable to polluted drinking water are numerous in the literature. Usually these outbreaks are followed by cases of typhoid fever; occasionally they consist only of acute intestinal distress without the subsequent development of cases of typhoid fever. A series of such epidemics in the Ohio River valley was investigated recently by Veldee.[1] He stated, "There is no epidemiological evidence to show that the ailment was produced by a viable organism contained in the water supply, unless we are to assume the sudden appearance of some bacterium or virus whose presence was not indicated by the established methods of water analysis."

The possibility of the presence of some such microorganism in sewage-contaminated water was investigated. This report concerns the isolation and preliminary study of a form of viable organism from polluted river water, the filtrates of which were apparently sterile when tested by ordinary bacteriological methods.

A sample was taken from the Chicago River at a point where it was heavily polluted with domestic sewage. After filtration through

[3] Rothschild, M. A., and Kissin, M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 577.

[1] Veldee, M. V., *Am. J. Pub. Health*, 1931, **21**, 1227.

paper, the water was filtered through a tested Berkefeld N filter and the filtrate collected aseptically. Cultures of this filtrate yielded no growth in solid or liquid media. Serial plating of the filtrate was begun, using the Hauduroy technique[2] as modified in this laboratory.[3] The method consists essentially of surface inoculation of litmus lactose agar plates, incubation for 48 hours, and inoculation of subsequent plates with the washings from the surface of the preceding plate. The inoculations are confined to a square area in the center of the plates, and the capillary pipettes and broth to be used in the operation are sterilized by autoclaving. Three series of plates were used; the first plate of each series was inoculated with a different amount of river water filtrate. The time of appearance of growth in each series of plates varied inversely as the amount of inoculum used on the first plate. For example definite signs of growth appeared in the 7th serial transfer of the set of plates which received 8 drops of the original filtrate. The set of plates derived from an original inoculation of 6 drops of filtrate first showed growth in the 8th serial transfer; and the set inoculated at the beginning with only 4 drops of filtrate did not show growth until the 9th serial transfer. Each of the 3 series of plates was handled in exactly the same manner, with the exception of the amount of filtrate used on the first plates. Aside from the time of appearance, the growth in all 3 series was apparently identical; this fact tends to rule out the probability that we were dealing with chance contaminants. That the appearance of visible growth was delayed in the series of plates receiving smaller inocula, also adds support to the conception that this growth was derived from the filtrate and not from extraneous sources.

The first sign of growth was a thin grayish film appearing over the inoculated area of the plates, and confined to this area. Under the low power of the microscope this was seen to be a confluent patch of growth without discernible structure. Smears showed small Gram negative rods with Gram positive granules scattered throughout, or single granules at each end of the rod. Transferring was continued as above to increase the growth. In the subsequent cultures the growth increased in amount, as evidenced by the thicker film, but was always confluent. In attempting to get isolated colony formation by streaking some of the material on plates by means of a wire, it was found that growth took place in only the more heavily inoculated areas and was confluent here also.

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[2] Hauduroy, Paul, *C. R. de la Soc. de Biol.*, 1927, **97**, 1932.

[3] Ryan, V. M., and Arnold, L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 899.

Growth in fluid media was slight; no fermentative reactions could be elicited in dextrose, mannitol, xylose, arabinose, saccharose, or maltose. Lactose broth became slightly acid in 10 days, without gas formation. The organisms were non-motile. Each of 2 rabbits were inoculated intravenously with 1 cc. of emulsion from the 13th serial plate; in one case the organisms were living, in the other, killed by heating at 65°C. for an hour. No effects were apparent in the animals, and the inoculations were repeated at intervals to produce agglutinating serum.

After 5 injections of 1 cc. each, the serum agglutinated the homologous organisms in a dilution of 1:640. The serum was also tested against various strains of typhoid, paratyphoid, colon, Shiga, and Flexner dysentery bacilli. No agglutination was noted except in the case of one strain of Flexner dysentery bacilli, which agglutinated in a serum dilution of 1:80. Absorption agglutination tests showed that the agglutinins for the Flexner strain could be completely removed without lowering the titer of the agglutinins for the river water organism.

This feebly growing, biochemically inert organism, occurring in a filterable form in polluted water, may have some sanitary significance, especially in view of the fact that it appears to be antigenically related to a known producer of dysentery. Repetition of these experiments, and further studies, including animal feeding tests are in progress.

6384

Skin Reactivity of Mothers and Infants to Gonococcus Vaccines.*

ALFRED J. KOBAK AND JOSEPH GREENGARD. (Introduced by F. H. Falls.)

From the Departments of Obstetrics and Gynecology, and Pediatrics, University of Illinois College of Medicine.

The skin reactivity of the newborn and young infant to intradermal injections of specific and non-specific substances has been studied by many investigators. Their results have invariably indicated that the skin in early life responds poorly or not at all to substances that cause marked reactions in adults and older children. Thus the dermal response to antigens prepared from the diphtheria

* We wish to thank Dr. Russel D. Herrold for the gonococcus antigens used in this study.

bacillus, scarlet fever streptococcus, tubercle bacillus and Staphylococcus aureus is comparatively negative in young infants but nevertheless consists in large reactions in adults (Ruh and McClelland,[1] Cook,[2] Kobak and Pilot[3]). It was likewise shown that protective antibodies are not the factors in negative dermal reactivity in early infancy. The basis for this is believed to be the lack of development of a mechanism of skin reaction to intradermal irritants (Friedberger and Heim,[4] Tschertkow[5]). The skin is also sluggish in its response to non-specific antigens and dermal irritants during infancy (Friedberger and Heim,[4] Adelsberger[6]). The phenomena of inert dermal response in early life was confirmed in laboratory animals by Freund[7] who used tuberculin (in guinea pigs) and vaccines of virulent pneumococci (in rabbits). This investigator also noted that the state of development of many immune bodies was likewise much less in the young rabbit as compared to the older animal.[8]

Staphylococcus aureus filtrate and vaccine were used by Kobak and Pilot to investigate the effect of intradermal reaction to bacterial products from organisms to which babies are clinically very susceptible. They obtained the staphylococci from boils and cases of Pemphigus neonatorum. For this study a series of mothers and their newborn babies, and another series of infants of various ages were injected. The results were quite similar to those where the diphtheria, scarlet fever and other antigens were used, namely, a high proportion of reactivity in mothers and a very low proportion in infants in whom the percentage of positive reactions gradually increased as older babies were tested. At the time this work was being completed we were inquiring into the effects of gonococcus antigens. The clinical susceptibility of infants to this organism is well known. Herrold[9, 10] prepared toxic filtrates and vaccines from the gonococcus which gave marked dermal reactions.

Our patients for this study were obtained from the Obstetrical

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[1] Ruh, H. C., and McClelland, J. E., *Am. J. Dis. Child.*, 1923, **25**, 59.

[2] Cooke, J. V., *Am. J. Dis. Child.*, 1927, **34**, 969.

[3] Kobak, A. J., and Pilot, I., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 584.

[4] Friedberger, E., and Heim, F., *Deut. Med. Wochenschr.*, 1929, **55**, 1932.

[5] Tschertkow, L. Z., *Z. f. Immun. forschg.*, 1929, **64**, 407.

[6] Adelsberger, L., *Z. f. Kinderh.*, 1927, **43**, 373.

[7] Freund, J., *J. Immun.*, 1929, **17**, 465; *J. Exp. Med.*, 1931, **54**, 171.

[8] Freund, J., *J. Immun.*, 1930, **18**, 315.

[9] Herrold, R. D., *J. Am. Med. Assn.*, 1925, **84**, 361.

[10] Herrold, R. D., Hoffman, S. J., and Blatt, M. J., *Ven. Dis. Inform.*, 1930, **11**, 397.

Department of the University of Illinois where 26 mothers and their babies were studied, and from the St. Vincent's Infant Asylum where 94 babies ranging from 2 weeks to 2 years were studied. We found that the gonococcus filtrate gave marked reactions in adults but progressively deteriorated and the skin response rapidly decreased in a short time. We, therefore, have employed the more stable vaccine from diluted broth culture. The dosage used was the amount that produced an average reaction of from 3 to 4 cm. in adults. This dosage was likewise given to all infants tested. The injected area was measured 24 hours later and the length and width were noted. The average of these 2 dimensions was the size recorded. Reaction consisted usually of an elevated area of hyperemia which reached its height in 24 hours and gradually disappeared in 3 to 5 days. All adults studied gave large reactions, and the average of the puerperal mothers was 3.6 cm. Reaction in the infants was both of a lesser degree and in smaller proportions. The average size and percentage of positive cases increased when older babies were injected. The findings (Table I) are in accord with the observations on staphylococcus, diphtheria and scarlet fever streptococcus skin studies reported by others.

TABLE I.
Skin Reactions to Intradermal Injections of Gonococcus Vaccine.

	No. Tested	Reactions Positive	Reactions Negative	% Positive	Average Size of Reactions*
Mothers					
Several hr. to 10 days puerperium	26	26	0	100.0	3.6
Newborn Babies					
Few hr. to 10 days	26	15	11	57.7	1.2
Infants					
2 weeks to 3 mo.	12	4	8	33.3	1.17
3 to 6 mo.	30	17	13	56.6	1.5
6 to 9 mo.	17	11	6	64.7	1.3
9 to 12 mo.	12	10	2	83.3	1.6
12 to 24 mo.	23	16	7	70.0—	1.8

*Average of reactions includes only positive cases of each group.

Conclusions. The poor skin response to gonococcus antigens to which newborns and older infants are clinically more susceptible resembles that of other bacterial preparations similarly used. We believe this phenomenon ("anergy") to be due to an underdeveloped mechanism for dermal reactivity in early age.

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Effect of Gastric Mucin on Virulence of Bacteria in Intraperitoneal Injections in the Mouse.

W. J. NUNGESTER, A. A. WOLF AND L. F. JOURDONAIS.

From the Department of Bacteriology, Northwestern University Medical School.

While studying the effect of such substances as gelatin and gastric mucin on the phagocytosis of the pneumococcus in the peritoneal cavity of the mouse, it was noted that the animals receiving pneumoccocus suspended in mucin died sooner than the control animals receiving the same dose in saline. In further studies we have used samples of dried commercial gastric mucin sterilized, or at least made innocuous to mice by heating at 140°C. for 1 hour *in vacuo* or by allowing it to soak in 80% ethyl alcohol for 1 week, then removing the alcohol by decanting and evaporating *in vacuo* at 40°C. Four gm. of mucin so prepared were added to 100 cc. of saline and this served as the menstrum in which was suspended an indicated dilution of a 24-hour glucose broth culture of the organism under examination. One cc. of the resulting suspension was then inoculated into the peritoneal cavity of a mouse.

The results obtained in the case of the pneumococcus II, as indicated in the table, show that death occurs much sooner in animals injected with organisms suspended in mucin than in those receiving a saline suspended inoculum. This culture also was lethal in a higher dilution when inoculated with mucin than when saline was used. A number of experiments with a hemolytic streptococcus culture were performed over a 5-month period during which mice were incubated in pairs with the mucin and saline suspended organisms respectively. The results were quite constant and, as seen from the summary of these experiments in the table, there is both an increase in the number of deaths and a decrease in the time of death for mice injected with the streptococcus suspended in mucin. We never succeeded in killing mice by injecting intraperitoneally 1 cc. of a 1:10 dilution of a recently isolated *Staphylococcus aureus* culture suspended in saline. When this culture, however, was suspended in mucin and injected, death usually followed in less than 20 hours. It was only after the culture had been in the laboratory several months that it failed to kill constantly when suspended in mucin.

Various sites of inoculation have been tried and this work is still under investigation. When the inoculation is made in the tail vein

TABLE I.

Organism	Mucin No. Mice Inoc.	Mucin No. Mice Dead	Mucin Av. Time Hr.	Saline No. Mice Inoc.	Saline No. Mice Dead	Saline Av. Time Hr.
Pneumo. 1:10	6	6	17	6	6	35
1:100	8	8	20	6	6	97
1:1000	4	4	20	4	4	63
1:10,000	2	2	32	2	0	—
1:1,000,000	2	0	—	2	0	—
Strept. 1:10	31	30	21	31	18	58
Staph. 1:10	12	7	16	12	0	—

of a mouse the effect of the mucin is not apparent. Of 6 mice injected intravenously with 0.5 cc. of streptococcus in mucin but 2 died and these in 120 hours. Similar results were obtained when the organisms were injected in saline whereas the same dose in mucin injected intraperitoneally killed 5 out of 5 mice in 17 hours and 4 out of 5 mice in 69 hours when the organisms were suspended in saline. Subcutaneous inoculations of mucin suspended inoculum appear to be no more lethal than similar doses of the organisms suspended in saline.

We have determined that agar, starch or gelatin which may offer a similar temporary protecting coat to the organism against the defensive mechanism of the host also tend to increase the virulence of the bacteria but not nearly as effectively as mucin. Acidulated gelatin (pH 3.0) was used as a means of irritating the peritoneum but did not hasten the invasion of the bacteria. Mice injected first with India ink to block the fixed phagocytic cells did not increase the susceptibility of the animals. If 1 cc. of 4% mucin is injected not longer than 5 or 6 hours before the introduction of 1:10 cc. of streptococcus culture into the peritoneal cavity of the mouse, the effect of mucin on the virulence of the organism is obtained.

6386

Peripheral Blood Chemistry Changes After Unilateral Lumbar Sympathectomy.

PAUL E. MC MASTER. (Introduced by Lester R. Dragstedt.)

From the Department of Surgery, University of Chicago.

Few studies have been reported on peripheral blood chemistry changes subsequent to sympathectomy. Fontaine and Jung[1] did

[1] Fontaine, R., and Jung, A., *La Presse Med.*, 1928, **36**, 1079.

unilateral cervical sympathectomies on rabbits and then produced experimental wounds of both ears. They found a higher pH in the exudate from the wound on the sympathectomized side compared to the "normal". Beattie, *et al.*,[2] studied chemical changes in hind leg muscles after unilateral lumbar sympathectomy. They found within 6 weeks of operation, (1) a rise in the water content, (2) a slight swing in the reaction to the alkaline side and (3) a lower lactic acid value on the sympathectomized side. Büttner,[3] doing similar work, found an increase in glycogen, lactic acid and ammonia and a decrease in phosphorus. Britton,[4] however, found a decreased glycogen content in the muscles while Dworken[5] and coworkers found no essential differences in muscle glycogen.

In the work reported here studies were made on the return blood flow from the hind legs after unilateral lumbar sympathectomy, which included at least 3 ganglia and intervening chain. Dogs were used and aseptic technique employed throughout. Blood examinations were made from 2 days to 7 months post-operative. The blood was drawn simultaneously from both femoral veins at the same level, when the dogs were perfectly quiet and relaxed.

Thirteen dogs were used and 33 CO_2 determinations were made. In CO_2 estimations made 2 days after operation, no essential changes were noted in the 2 sides. Twenty-three determinations were made from one week to 5 months after operation and the average CO_2 content on the sympathectomized side was 2.1 volumes % lower than the non-sympathectomized side. These differences varied from 1.5 to 5.2 volumes %. This difference in CO_2 disappeared after 5 months, although one dog showed no changes after 6 weeks. One, examined at 6 and 7 months after operation, has shown no CO_2 differences, although marked changes were present previously.

Five oxygen determinations were made at post-operative intervals of 10 days to 4 months. There was in each case a greater O_2 content on the sympathectomized side ranging as high as 1.77 volumes % and averaging 0.96. One examination made 2 days after operation showed no difference.

Lactic acid and sugar determinations were made but no differences were found on the 2 sides even when definite CO_2 changes were present.

Hematocrit readings made simultaneously when drawing blood

[2] Beattie, F. J. R., Beattie, M. K., and Milroy, T. H., *J. Phys.*, 1930, **69**, 364.
[3] Büttner, H. E., *Ab., Am. J. Phys.*, 1929, **90**, 304.
[4] Britton, S. W., *Am. J. Phys.*, 1930, **93**, 213.
[5] Dworkin, S., Baeq, Z. M., and Dill, D. B., *Am. J. Phys.*, 1931, **96**, 308.

for CO_2 were higher on the sympathectomized side although red blood cell counts showed no essential differences on the 2 sides.

As the lactic acid and sugar estimations showed no differences, the changes in blood gas volumes are probably not of metabolic origin. Following lumbar sympathectomy there is a release of normal vascular tonicity. As this tonicity offers some resistance to blood flow normally, the removal, by sympathectomy, of this factor would permit a greater blood flow through the extremity. This has been demonstrated by Britton. Hence with a greater blood flow through the extremity, each unit volume of blood would have to carry less CO_2 and give off less O_2. Physiological readjustments apparently take place after a few months, restoring the sympathectomized side to an essentially normal condition.

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Effect of Sympathectomy on Bone Repair.

PAUL E. MC MASTER AND N. W. ROOME.
(Introduced by Lester R. Dragstedt.)
From the Department of Surgery, University of Chicago.

The effect of unilateral lumbar sympathectomy on the healing of bone defect has been studied experimentally in the following manner. Dogs were used throughout. A left lumbar sympathectomy, including at least 3 ganglia, sometimes 4, and the intervening chains, were excised from the sacral promontory upwards. Immediately following this procedure, equal fragments, approximately 1.5 mm. in length, were resected subperiosteally from the upper end of both fibulae. This method for studying bone repair was chosen in preference to simply fracturing the bones, as repair can be studied more satisfactorily by roentgenograms. Also the fibulae were chosen as they are not essential to weight bearing, hence splints and casts were not used. Healing was determined by roentgenograms and was considered complete when callus had completely bridged the gap between the bone ends.

Seventeen dogs were used. Seven developed infection of the fibular wounds and were sacrificed. Six of the remaining 10 presented an unexpected complication of fibular bone absorption rather than healing, and will not be discussed in this paper.

Of the remaining 4 one dog died of pneumonia 7 weeks after

operation. At the time of death there was more callus on the non-sympathectomized side, although the bone defect was not completely healed.

Complete healing occurred in the other 3, the average time being 12 weeks. In each, there was a more rapid healing on the non-sympathectomized side, averaging 3 weeks sooner.

Fibular fragments were excised from another dog, in which only a left lumbar sympathectomy had been done 6 months previously. No difference was noted in the healing time, between the sympathectomized and the non-sympathectomized sides. In this case the effects of the sympathectomy had probably worn off at the time the bone fragments were removed.

These results are in contradiction to the clinical experiences of Colp and Mage.[1] These authors did periarterial sympathectomies, in Scarpa's triangle, in acute and potentially delayed fractures and stated that in these cases compared with a "control" series, the union was more rapid by about 2 weeks. Pearse and Morton[2] concluded from experimental work that if the sympathetic nervous system has any effect on hastening osteogenesis, it is a very slight one.

Experiences, both clinically and experimentally, have shown that more pronounced and longer lasting effects result from lumbar sympathectomy than from periarterial sympathectomy. Yet with the increased arterial hyperemia resulting from lumbar sympathectomy, this has not been shown experimentally to hasten the repair of bone. Consequently there is considerable doubt as to the advisability or the beneficial effect, of doing any type of sympathectomy in an attempt to hasten bone repair in clinical cases.

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[1] Colp, Ralph, and Mage, Sigmund, *J. Am. Med. Assn.*, 1931, **97**, 1069.

[2] Pearse, H. E., and Morton, J. J., *J. Bone and Joint Surg.*, 1931, **13**, 68.

6388

Accessory Regulatory Mechanism of Respiration.*

D. B. WITT, L. N. KATZ AND L. KOHN.

From the Cardiovascular Laboratory, Department of Physiology, Michael Reese Hospital, Chicago.

The investigations of Heymans,[1] Schmidt,[2] Cromer and Ivy[3] and others have emphasized the importance of the nervous control of respiration. We wish to report the effects on respiration of denervating the sensory end organs in the aorta, carotid sinus and lungs.

In brief, in 25 dogs, under moderate morphine-barbital anesthesia, in which the region of the carotid sinuses was denervated, respiratory death occurred in 10 either immediately or within a few minutes. Eleven of the remaining 15 dogs died following section of the vagi near the jugular foramen. Similar deaths were obtained in 1 of the 2 rabbits and the 2 cats used. Of 4 dogs under ether anesthesia, 1 under moderate anesthesia died following denervation of the carotid sinus regions and vagotomy. The other 3, under light anesthesia, survived the procedure. Five of the 29 animals under barbital anesthesia also survived both procedures. However, in all the animals that survived a prolonged apnea was obtained.

These results suggested that the effect of denervation depends on the sensitivity of the respiratory center. The following observation strengthened this impression: a dog under moderate morphine-barbital anesthesia, after sectioning of the vagi and denervation of carotid sinus regions, developed protracted apnea accompanied by the usual drop in blood pressure. Immediately after apnea appeared, the tracheal cannula was connected with a spirometer containing 10% CO_2. Manual compression of the chest 3 times, one minute after the occurrence of apnea (so are to force the 10% CO_2-air mixture into the lung alveoli) caused an immediate resumption of breathing and restoration of blood pressure to its previous level. This state continued after ordinary air was substituted for the CO_2 rich mixture. The resumption of breathing in this case was not due to the manual compression because this procedure was tried with ordinary air without effect in many of the other dogs that died.

* Aided by the Emil and Fanny Wedeles Fund of the Michael Reese Hospital for the Study of Diseases of the Heart and Circulation.

[1] Heymans, C., Bouckaert, J. J., and Dautrebande, L., *Pflüger's Arch. f. d. gesamte Physiol.*, 1932, **230**, 283.

[2] Schmidt, C. F., *Am. J. Phys.*, 1932, **102**, 94.

[3] Cromer, S. P., and Ivy, A. C., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 565.

These results indicate that the respiratory center is under the tonic control of the end organs of the carotid sinus region and of the aortic region, as well as those located in the lungs. These results suggest, furthermore, that this tonic activity is particularly important when the respiratory center is in a depressed state and also that the removal of this tonic influence at such a time may lead to respiratory death.

6389

Experimental Spontaneous Peptic Ulcer.

PAUL E. MC MASTER. (Introduced by Edmund Andrews.)

From the Department of Surgery, University of Chicago.

In this work physiological processes other than the occurrence of peptic ulcer were being studied after jejuno-colostomy. A resulting and unexpected high incidence of peptic ulcer, however, has led to a study of this condition. All operations were performed on healthy adult dogs under morphine-ether anesthesia, using aseptic technique. Through a right paramedian incision the jejunum was isolated and sectioned approximately 25 to 30 cm. distal to the ligament of Treitz. Both ends were inverted. A side-to-side anastomosis was then made between the proximal jejunal stump and the caecum or ascending colon. At no time during the procedure was the stomach, duodenum or upper abdomen handled or explored, thus eliminating the element of trauma. The abdomen was closed. For 3 days after operation the animals were given intravenous saline only. Fluids and solid food were then given by mouth in gradually increasing amounts, until they were receiving the regular stock diet.

Seven dogs have died at intervals, longer than 8 days after operation. In 6 of these 7 animals, autopsy revealed on gross examination acute to more chronic forms of peptic ulcers. In one dog autopsied 8½ days after operation an acute gastric ulcer, 2x2 mm., with destruction of the mucosa was found 4 cm. proximal to the pylorus. Two other animals, having died 14 and 27 days after operation, showed gastric ulcers in the pre-pyloric region. The first was an acute ulceration of the mucosa with rounded margins, measuring 2x2 mm., and the other was a large ulcer 1x0.6 cm., having indurated and rolled margins and penetrating deeply into the stom-

ach wall. Near this last ulcer was a second small acute ulceration 2x1.5 mm.

Two dogs died of perforated duodenal ulcers, 39 and 53 days respectively after operation. In the first one the duodenal ulcer was 2 cm. distal to the pylorus and measured 1.4x0.8 cm. There was an associated acute gastric ulcer, 1.5 cm. proximal to the pylorus, 4x6 mm. in size. In the second case the duodenal ulcer was 2.5 cm. distal to the pylorus and measured 2x2.5 cm. In this case there was also an associated prepyloric ulcer 1x0.5 cm., the margins being indurated and rolled, with the appearance of being chronic.

One dog died 144 days after operation and a chronic indurated duodenal ulcer 1.2x0.8 cm. was found 2 cm. distal to the pylorus. The seventh dog died of pneumonia 40 days after operation, but revealed no peptic ulcer at autopsy.

There were no ulcers at the anastomotic rings and not other gastro-intestinal pathological findings, except in the seventh dog, in which 2 large acute ulcers were present in the colon, 8 cm. distal to the anastomosis.

In 2 dogs a duodeno-colostomy was done and they lived 22 and 28 days respectively. No peptic ulcers were present in these at autopsy. In several dogs that received the same care and food as the above dogs, but which died from other experimental procedures, no peptic ulcers were found.

Anorexia, vomiting and tarry stools occurred in the dogs with peptic ulcer. All the dogs in the series lost considerable weight. There are 2 factors accountable for this: first, a decrease of bowel absorptive space, and secondly, refusal of food by the dogs after ulcer symptoms developed.

Studies are being made to determine a specific etiological factor for the occurrence of peptic ulcer after this procedure.

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A Coagulo-Flocculation Test for Malignant Tumors.

EMIL WEISS. (Introduced by Lloyd Arnold.)

From the Department of Pathology, Bacteriology and Preventive Medicine, Loyola University School of Medicine, Chicago.

The study of protein fractions of normal serums, those in various diseases, and malignant tumors led to the development of a diag-

nostic procedure for malignant tumors. The essential constituents are: (1) Blood serum inactivated at 55° C. for ½ hour, which should be neither contaminated nor hemolized. (2) The antigen is prepared by extracting finely ground beef heart with 95% alcohol in the ratio 1:10 for 3 days at 37° C. and overnight at room temperature and then filtering. (3) A ½% watery solution of sodium sulphate serves as antigen diluent.

The serums are diluted before their use in the test according to the percentage of hemoglobin. Dare's hemoglobinometer is used as a standard, and to the percentage of hemoglobin obtained 10 is added and the sum is divided by 20, which gives the dilution for the respective serum. (For instance, the reading was 70% plus 10 = 80 ÷ 20 = 4. The dilution of the serum in this case would be 1:4.) Where the Dare reading is 45% or less, the serums should be diluted only to 1:3. All serums are diluted with the antigen—sodium sulphate mixture up to 1:3, and if a further dilution is necessary it is done with distilled water.

Titration of antigens is carried out as follows: Increasing amounts of undiluted antigen (.16 cc., .18 cc., .20 cc., .24 cc., .26 cc., etc.) are placed in the corresponding tubes of 2 rows, (each with 8 tubes). In each tube of the first row 0.6 cc. of the diluted malignant serum are added and each tube of the back row received 0.6 cc. of the diluted normal serum. The serums (0.2 cc.) when added to the antigen-sodium sulphate mixture (0.4 cc.) are diluted 1:3: if the serum requires a further dilution, it is done with distilled water before the addition of the serum. The tubes are thoroughly shaken and then placed in a water bath for 5 minutes at 55 C. After the incubation each tube is diluted with 5 cc. of distilled water, incubated for ½ hour at 55 C., and the results recorded. The largest amount of antigen which causes only turbidity in the syphilitic tube and a distinct flocculation in the malignant tube is selected as the proper amount for the test (= titre). The titrated amount of antigen should be also tested with syphilitic, jaundice and anemic serums. The titre remains the same for an indefinite period if the antigen is properly preserved.

The routine test: Wassermann tubes are placed in 2 rows in the racks. The tubes of the first row are used for the main test with the unknown serums and also for the malignant, syphilitic, jaundice and anemic controls. The last tube in the first row contains the antigen control. The tubes in the second row serve as the serum controls for the unknown serums and also for the malignant, syphilitic, and jaundice and anemic serums. The titrated amount of the

diluted antigen is placed in each tube of the first row. The corresponding amount of a 2% solution of sodium sulphate is placed in all tubes of the second row. Six-tenths cc. of each diluted serum are added to one tube in the first row and an equal amount of the same serum to the tube behind in the second row. Six-tenths cc. of a ½% solution of sodium sulphate (instead of serum) are added to the antigen control. The procedure for serums which require a higher dilution than 1:3 is described above (see antigen titration). The ingredients of the serum controls should be the same as those used for the unknown serums. All tubes are then shaken and placed in a water bath at 55°C. for 5 minutes. After the incubation each tube is diluted with 5 cc. of distilled water, again incubated for ½ hour at 55°C., and the results read. If the required amount of the unknown serum is not available, the test may still be performed successfully if the remaining constituents for the reaction are decreased proportionately.

Controls: The following controls are necessary each time the test is carried out: (1) antigen control, (2) serum control (each serum should have a serum control), (3) malignant, syphilitic, jaundice and anemic controls.

Interpretation of the results: The controls should be examined before making readings of the unknown serums. The malignant control should show a thick layer of coagulated serum floating on the surface of the salt solution, which contains many large floccula. All other controls should remain uniformly turbid. One tube is read for each unknown serum. Tubes with a distinct flocculation or showing in addition a layer of suspended coagulated serum on the surface of the saline are read as strong positive. Tubes with a fine flocculation are read as weak positive. Tests with doubtful flocculations are repeated. Uniformly turbid tubes are read as negative.

This test applies to all types of malignant tumors. The statistical data concerning the sensitiveness of the test include 179 malignant tumors with approximately 85% positiveness, while 154 cases of various diseases and 105 cases of benign tumors give non-specific reactions in approximately 2% of the cases.

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A Coagulo-Flocculation Test for Malignant Tumors. (Studies on Antigens.)

EMIL WEISS. (Introduced by Lloyd Arnold.)

From the Department of Pathology, Bacteriology and Preventive Medicine, Loyola University School of Medicine, Chicago.

In these studies the following tissues were examined: wet human heart and liver, wet and dry beef heart, liver, kidney, brain and muscle. Three main types of antigens were employed:

Plain alcoholic extract. The wet tissues were extracted with 95% alcohol in the ratio 1:10 for 3 days at 37°C. and kept over night at room temperature. These extracts were then filtered. Dry tissues were extracted in the ratios 1:10, 1:25, 1:50, and 1:100 at 37°C., room and ice box temperature. The duration of extraction was the same as stated for the wet tissues.

Tractional extracts. They were prepared from the above dry tissues. Ten grams of the powderized tissues were extracted with 150 cc. of acetone and likewise with ether, chloroform, benzol and xylol. These flasks were kept at 37°C. for one hour, the extracts were then filtered and evaporated to dryness. The residue was redissolved in 50 cc. of 95% alcohol. Similarly extracts were prepared at ice box and at room temperature.

Secondary extracts. As in the preceding method 10 gm. of the respective dry tissues were extracted with 150 cc. of acetone for one hour at 37°C. and likewise with ether, chloroform, benzol and xylol. The extracts were then filtered and discarded, while the tissue residue was dried at 37°C. for one hour or for a longer time if necessary. Each dried tissue was extracted with 50 cc. of 95% alcohol for 3 days at 37°C. and then over night at room temperature. These extracts were again filtered. Similar extracts were prepared at ice box and at room temperature.

The usefulness of the above extracts with regard to the test can be summarized as follows: Beef heart appears to be the most suitable tissue for extraction of lipoids and is not exceeded in this respect by any other tissue. Wet beef heart is preferable to dry one, which renders a more concentrated extract, thus narrowing the specific zone. This can be partially overcome by extracting the dry beef heart with comparatively large amounts of lipoid solvents. By using the wet beef heart, the solvent becomes diluted by the tissue fluids and in this manner its extractive power becomes greatly

decreased. The secondary extracts do not possess any advantages over the plain extracts. The fractional extracts, which seem to be useful actually give the effect of the solvents, their specific zone being slightly narrowed by the lipoids they contain. The lipoids alone in the useful extracts cause such a turbidity in the tubes that even for this reason only their usefulness appears to be a very slight one.

Cholesterol lecithin and a number of alcohol soluble gums (copal, mastic, gamgabe, tragacanth, chicle, benzoin, damar, sandarc, shellac white and orange, elemi, guaiac, camphor, catechu and kino) were examined concerning their usefulness in place of lipoids or in combination with lipoids; their value was found to be very slight as compared with lipoids. Among the lipoid solvents ethyl-alcohol and methyl-alcohol were the most satisfactory ones. The influence of time and temperature of extraction is of far less importance in the extracts for malignant tumors than observed in various extracts for the Wassermann test or precipitation test for syphilis.

Ratio 1:10 *between tissue and the extracting fluid*, for wet beef heart and 95% alcohol appears to be the most satisfactory. The same ratio applies to plain alcoholic extracts from dry tissues made up at room or ice box temperature. For dry tissues extracted at 37°C. the best ratio was 1:100. Similar results were obtained with the secondary extracts: if the second extraction was carried out at 37°C., the best ratio was 1:100, while the extraction made at room temperature gave the best results in the ratio 1:10. The fractional extracts did not show much variation in titer, whether the lipoids were redissolved at room temperature or 37°C., whether the ratio was 1:10 or 1:100. The extracts are most satisfactorily preserved if they are kept in dark colored bottles in a dark place at a temperature not lower than that at which they were prepared.

As a result of these studies alcoholic beef heart extracts are found to be the most satisfactory ones. Their preparation is as follows: 1. Plain wet alcoholic beef heart extract is prepared by grinding fat free beef hearts and extracting them with 95% alcohol in the ratio 1:10 for 3 days at 37°C. and over night at room temperature and then filter. 2. Plain, dry alcoholic beef heart extract is prepared by extracting beef heart powder with 95% alcohol in the ratio 1:10 for 3 days at room temperature and then filter. Similar results were obtained if the extraction in the ratio 1:100 for 3 days at 37°C., overnight at room temperature and then filter.

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Studies on Dissociation of Certain Paratyphoid Bacilli: Rôle of Variants in Precipitation of Calcium Sulphite.

MARY E. CALDWELL. (Introduced by Edwin O. Jordan.)

From the Department of Bacteriology, University of Chicago; and the University of Arizona.

In the course of certain bacteriophage studies, cells, thought to be a type of variant, have been found which have the ability to precipitate calcium sulphite. The technique employed and the detailed results, together with a review of the literature, will appear in a forthcoming publication. Since these presumable variants gave rise to a daughter-colony-like structure, within which sulphite crystals were incorporated, they may be termed thiosomes. These sulphite-containing bodies have apparently not been reported previously nor has microchemical technique been resorted to in studies of this type.

On "Bacto-" agar plates, typical thiosomes are small (0.005-0.015 mm.), opaque bodies which are superimposed upon and at times in the medium surrounding ordinary colonies. They are black by transmitted light, white by reflected light. show extinction with crossed nicols and are iridescent with polarized light. Microchemical technique employed for the detection of cations and anions present revealed crystals typical of Ca^{++} and SO_3^{--}. Thiosomes were originally observed associated with *Salmonella schottmülleri* colonies obtained from sealed filtrates which had become opalescent or slightly cloudy; from certain daily broth transfers, when no filtrations intervened, of *Escherichia coli, Eberthella typhi, Salmonella paratyphi, Salmonella schottmülleri* and *Salmonella paratyphi,* Type C; and in association with an atypical strain of *E. coli* obtained from a case of cervicitis. Under proper experimental conditions, thiosomes were produced at will from the above named cultures. Atypical thiosomes, which more closely simulated daughter-colony-like structures and which were devoid of blackness, were not polarized and never occurred outside the colonies, were found upon a synthetic medium and also upon ordinary fresh veal infusion agar plates. Media used contained several concentrations of NaCl and $CaCl_2$ and mammalian Ringer's; media to which no salts were added were also used. A distinction must be made between fresh veal infusion and dehydrated "Bacto-" veal infusion; a sample of the latter contained 1.5 mg. of calcium per liter, while the former showed only a trace.

Evidence that the fundamental structure of a thiosome is colony-like appear as follows: (1) Atypical thiosomes resemble the opaque ones in size, in structure, in position on the colonies and in their irregular occurrence. With polarized light atypical thiosomes do not appear to be of a crystalline nature. (2) Lack of any definite pattern of sulphite crystals when numerous and, when few in number, their formation on the initial streak of the plate only, suggest the presence of scattered variant cells. (3) Intermittent occurrence of thiosomes during serial transfers in the same lots of media to which either no salts or NaCl had been added; also, variations in numbers of thiosomes in the control $CaCl_2$ media. (4) The association of thiosomes with S and R colony types. (5) The development on the part of some thiosomes of scalloped margins which have either lost their blackness or are less opaque. (6) By application of suitable reagents, $CaSO_3$ crystals may be dissolved and leave a colony-like structure devoid of any blackness.

Experiments, under way at the present time, are designed to attempt the cultivation of variant cells from these sulphite-containing bodies.

The possibility that certain variant bacterial cells may have the ability to precipitate calcium and hence be of some significance in the formation of certain concretions in the animal body is discussed.

Pacific Coast Section.

Stanford University, October 15, 1932.

6393

The Spinal Rabbit and Its Reflexes.*

J. C. HINSEY AND C. C. CUTTING.

From the Department of Anatomy, Stanford University.

Fifteen rabbits have been transected in the lower thoracic region and have been kept from 3 to 17 days. They ate rolled barley and lettuce and drank water in a normal manner. They defecated and urinated but it was found necessary to evacuate the bladder by pressure upon the abdomen to prevent excessive retention. Trophic disturbances were avoided by keeping the animals and cages clean.

They were observed from day to day for ipsilateral and crossed reflexes in the hind limbs. Ipsiflexion, crossed flexion and occasionally crossed extension were present 24 hours after transection. Crossed extension, however, did not usually appear till about the third day and sometimes later. At first it consisted of extension at the knee and flexion at the ankle and toes. This usually changed to extension at all joints. The Stütz positive reflex became well-developed about the third day. Rhythmic stepping and hopping did not usually appear till after 4 days and they became more pronounced and greater in amplitude from day to day. In the same animal, it was possible to elicit hopping at one time, then after a short interval stepping would be present. Some of these animals were able to support their weight in the standing position but this was more poorly developed than in the cat. In the rabbit, there is considerable resistance to passive flexion in certain positions which is not due to muscle contraction for it can be obtained in the dead animal.

It was possible to anesthetize with ether, then to dissect and isolate the gastrocnemius and anterior tibial muscles. The animal was then placed in a frame and the muscles prepared for recording reflex responses according to the method we have described.[1] It was then

* This study was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University.

allowed to recover from the anesthetic for about one hour before kymographic tracings of its responses were made. Thus it was possible to record the responses in an unanesthetized animal which was not at all disturbed by the procedure; in fact at times they ate carrots and lettuce during the progress of the experiment. The reflexes in these muscles were elicited by normal stimulation to the ipsilateral and contralateral limbs, and also by electrical stimulation of isolated nerves.

As a whole, the spinal reflex patterns in the rabbit resemble those in the spinal cat (Ranson and Hinsey,[2] Hinsey, Ranson and Doles,[3] and Hinsey and Cutting[1]). However, ipsilateral extension was much more highly developed and more regularly obtainable in the rabbit than in the cat. By touching the plantar surface of the foot, especially near the heel, it was possible to elicit regularly a tonic contraction of the gastrocnemius of the same limb. The quadriceps femoris also participated in the response. That exteroceptive stimulation elicited this ipsilateral extension was shown by the fact that brushing the hair on the heel produced this response. It disappeared following section of the tibial nerve at the ankle. The flexors relaxed during the ipsilateral extension and, as in the cat, we have obtained no support for cocontraction of the flexors and the extensors from Stütz positive stimulation in the spinal rabbit.

The responses produced by electrical stimulation were quite variable. In general it may be said that weak stimulation predisposed to crossed extension and stronger stimulation to crossed cocontraction. Frequency reversals were seen in the crossed responses. Very weak faradic stimulation of the tibial nerve at the ankle produced a postural ipsilateral extension. To our knowledge, this is the first time this response has been obtained with electrical stimulation in spinal animals. Stronger stimuli produced either pure ipsilateral flexion or cocontraction.

Rabbits have been found very suitable for the investigation of spinal patterns. They may be kept for long periods following the transection and they require comparatively little care. They are particularly well adapted for the recording of contractions in isolated muscles, because it is possible to do this without the depression of blood pressure that accompanies decerebration. We have recorded reflexes 9½ hours after the original dissection and they were as good at that time as earlier in the experiment.

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[1] Hinsey, J. C., and Cutting, C. C., *Am. J. Physiol.*, 1932, **102**, 183.

[2] Hinsey, J. C., Ranson, S. W., and Doles, E. A., *Am. J. Physiol.*, 1930, **95**, 573.

[3] Ranson, S. W., and Hinsey, J. C., *Am. J. Physiol.*, 1930, **94**, 47.

6394

A Search for Neurological Mechanisms in Ovulation.*

J. C. HINSEY AND J. E. MARKEE.

From the Department of Anatomy, Stanford University.

There is no doubt that the nervous system is involved in producing the ovulation which follows copulation in the rabbit. The observations here presented represent one phase of our attack upon this problem.

Ascheim and Zondek[1] found that a substance (Prolan) in the urine of pregnant women produces effects similar to the sex hormone in the anterior lobe of the hypophysis. Friedman[2] induced ovulation in the rabbit by the intravenous injection of the urine of pregnant women. The utility of this reaction depends upon the fact that ovulation does not occur spontaneously in the rabbit (Heape,[3] Ancel and Bouin,[4] Hammond and Marshall[5]). We have utilized the Friedman phenomenon to produce ovulation at will in the rabbit. Evidence has been presented that prolan and the sex hormone from the anterior lobe of the hypophysis are not identical (Parkes and Hill,[6] Reichert, Pencharz, Simpson, Meyer and Evans,[7] Evans, Meyer and Simpson,[8] Wallen-Lawrence and Van Dyke,[9] and Loeb[10]). We have been concerned in finding out whether or not any of the efferent pathways from the central nervous system are involved in the ovulation that follows the injection of pregnancy urine into the rabbit. Although ovulation has occurred in transplanted ovaries in many species including man (Knauer,[11] Grigo-

* This investigation was conducted with the aid of the Rockefeller Foundation Grant for Fluid Research in the Medical Sciences at Stanford University and a grant from the Sex Division of the National Research Council.

[1] Ascheim, S., and Zondek, B., *Klin. Wochenschr.*, 1928, **7**, 30.

[2] Friedman, M. H., *Am. J. Physiol.*, 1929, **89**, 438.

[3] Heape, W., *Proc. Roy. Soc. London*, (Series B), 1905, **76**, 260.

[4] Ancel, P., and Bouin, P. J., *J. de physiol. et de path. gen.*, 1911, **12**, 1.

[5] Hammond and Marshall, Reproduction in rabbits. 1925. Oliver and Boyd, Edinburgh and London.

[6] Parkes, A. S., and Hill, M. J., *J. Physiol.*, 1930, **69**, 23.

[7] Reichert, F. L., Pencharz, R. I., Simpson, M. E., Meyer, K., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 843.

[8] Evans, H. M., Meyer, K., and Simpson, M. E., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 845.

[9] Wallen-Lawrence, A., and Van Dyke, H. B., *J. Pharmacol. and Exp. Therap.*, 1931, **43**, 93.

[10] Loeb, L., *Endocrinol.*, 1932, **16**, 129.

[11] Knauer, E., *Z. f. Gynäk.*, 1896, **20**, 524.

rieff,[12] and Frank[13]), this is the first study in the rabbit of induction of ovulation in ovaries completely isolated from the influence of the central nervous system.

Virgin rabbits, weighing 2 to 3 kilos, were isolated in individual cages for 3 weeks. Ovulation was induced in each case by the injection of 8 to 10 cc. of the urine of pregnant women, and the ovaries were examined approximately 20 hours following the injection. An autopsy was done to control the experimental procedures in each of the experiments.

In a series of 5 animals transected at the level of the 10 T spinal cord segment, the 4 into which urine was injected 4 days following the transection, showed ovulation with 3 to 9 rupture points in the 2 ovaries. The one used as a control without the injection of urine showed no ovulation. In one rabbit sectioned at the 4 T segment and in 2 sectioned through the 5 T segments, the ovaries showed 6 rupture points in each case.

Ovulation with 5 rupture points was present in one animal in which the spinal cord was completely removed from 5 T to below the 3 L segment. The spinal cord was removed caudal to the 1 S segment so that all of the sacral nerves were sectioned in one animal and caudal to the 5 L segment in another. Six rupture points were found in the former and 5 in the latter following the injection of pregnancy urine.

Inasmuch as the experiments so far had shown that ovulation occurred even after complete excision of the thoracolumbar segments giving rise to the preganglionic thoracolumbar sympathetic fibers on the one hand and the total absence of the sacral segments of origin of the preganglionic sacral parasympathetic fibers on the other, it was deemed necessary to completely sever the nervous pathways from the central nervous system which might exert an influence upon the ovary. The experiments devised to accomplish this were performed in 3 stages. First, the thoracolumbar cord was completely removed under ether anesthesia. Three days later the animals were again anesthetized with ether and the sacral cord was removed. Following these 2 procedures, the animals ate, drank and defecated. They were in excellent condition. Bilateral section of the vagus in the neck was performed under ether anesthesia after another interval of 5 days. The urine was injected immediately after the last operation. Three animals were used and it seems advisable to describe the autopsy findings individually. In

[12] Grigorieff, W., *Z. f. Gynäk.*, 1897, **21**, 663.

[13] Frank, L., *Z. f. Gynäk.*, 1898, **22**, 444.

one animal in which 2 rupture points were present, the spinal cord was removed from the 4 T through the 2 L inclusive and caudal to the 6 L segment; both vagi were completely sectioned. In another in which 4 rupture points were observed, the cord removal extended from the 4 T through the 2 L segments and caudal to the 6 L segment; both vagi were sectioned. In a third animal, immediately after vagotomy, 10 cc. of urine from a non-pregnant woman was injected. Twenty-four hours later when an exploratory operation was performed, ovulation had not occurred. Immediately 10 cc. of pregnancy urine was injected. The animal died during the night but one follicle had ruptured. Autopsy showed that the spinal cord had been removed from the 4 T through the 2 L segment and caudal to the 5 L segment; both vagi were cut in the neck. Microscopic observations confirmed the occurrence of ovulation where rupture points had been observed grossly.

These experiments conclusively show that ovulation induced by the injection of pregnancy urine does occur in the complete absence of the visceral afferent and efferent vagal pathways to the ovary, and of those which pass to and from the thoracolumbar and sacral segments of the spinal cord. The efferent pathways were certainly completely removed; thus rendering impotent the effect which any few remaining afferent pathways might exert.

6395

The Protein-Crystalloid Complex as an Antigenic Unit.*

ALTON C. KURTZ, HAROLD C. SOX AND W. H. MANWARING.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University.

It was implied in all classical theories of specific immunity that the alien protein molecule functions as a single antigenic unit, stimulating the production of a single antiprotein substance or function. Within the last decade, however, this early theory has been challenged, several European theorists denying the assumed antigenic unity and postulating that the protein molecule is little more than an immunologically inert mechanical "carrier" of potentially antigenic superficial crystalloids. Each of these superficial "determinants," "coefficients," or "unit characteristics," is pictured as func-

* Supported in part by the Rockefeller Fluid Research Fund of the School of Medicine of Stanford University.

tioning as a practically independent antigenic unit. There are numerous variants of this theory, differing mainly in the postulated degree of independence or interdependence of the sub-colloidal specificity determinants.

This concept is an apparently logical deduction from the "Landsteiner phenomenon," the well-confirmed fact that immunochemically non-related proteins are more or less homologized if conjugated with the same non-antigenic crystalloid. The resulting antiserums apparently have anti-crystalloid functions which can be specifically "absorbed" from such serums.

A critical examination of published data of this type, however, has suggested a possible source of logical error, or at least of a distortion of the logical perspective. Medvecky and Uhrovits,[1] for example, studied the relative specificities of artificial protein-benzoyl conjugates. Quantitative analyses of all wash waters and other waste products in a duplication of their technic suggest that the average colloid of their end-products must have been the "carrier" of no less than 150 to 200 attached benzoyl radicals. Such a mechanical burying of the protein under a unit crystalloid must have given antigenic conditions rarely if ever duplicated in nature. We have, therefore, modified the quantitative relationships of their technic so that our average end-product is the "carrier" of not more than 20 to 30 benzoyl radicals. Typical relative specificities of 3 such partially benzoylated antigens are shown in Table I.

TABLE I.

Heterophile Relationships of Partially Benzoylated Natural Antigens.

Dilution of Stock Solution	Titration with Anti-BEW Precipitin			Titration with Anti-BHS Precipitin		
	BEW	BHS	BDS	BEW	BHS	BDS
1:320	+	+±	0	0	+++	++
1:640	+++	+	0	0	++++	++
1:1,280	++	+	0	0	+++	+±
1:2,560	++	+	0	0	+++	+
1:5,120	+±	±	0	0	++	+
1:10,240	+	±	0	0	++	+
1:20,480	+	0	0	0	+	+
1:40,960	±	0	0	0	+	+
1:81,920	0	0	0	0	±	±
1:163,840	0	0	0	0	0	±
1:327,680	0	0	0	0	0	±
1:655,360	0	0	0	0	0	0

BEW, benzoylated egg white; BHS, benzoylated horse serum; BDS, benzoylated dog serum; parallel titrations by means of anti-BEW and anti-BHS rabbit precipitin. 0.5 cc. 10% anti-serums, plus 0.5 cc. increasing dilutions of 7.5% stock solutions of the benzoylated antigens; incubator 2 hours, ice-chest over night; quantitative readings by means of a turbidity scale.

[1] Medvecky, A., and Uhrovits, A., *Z. f. Immunitätsforsch.*, 1931, **72**, 251.

It is seen that the 3 partially benzoylated antigens have no invariably common fractional identity, in spite of the presence of an equal number of benzoyl "coefficients" in each antigen. Moreover, the heterophile relationships suggested by titration with one antiserum are not the same as those suggested by a parallel titration with an antiserum of different specificity, both antiserums presumably containing the same antibenzoyl fractional antibody or function.

These data are almost impossible to harmonize with the suggested theory that each protein is but a mechanical "carrier" of independent superficial unit "determinants". They can be harmonized, however, with the classical postulate that each protein-crystalloid complex functions as a single antigenic unit.

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Emetic and Fatal Doses of Digitalis at High Altitudes.

A. J. LEHMAN AND P. J. HANZLIK.

From the Department of Pharmacology, Stanford University School of Medicine, San Francisco.

For over a hundred years, there has been some knowledge of changes in effects of drugs at high altitudes. However, some scientific studies, according to a summary of Loewy,[1] indicate conflicting tendencies. Nevertheless, it would seem that changes in certain pharmacological actions might be expected from known or assumed changes in circulatory and respiratory functions, although the degree or character could not be exactly predicted, owing to the adjusting influences of compensatory mechanisms. The interest attached to any possible changes is both fundamental and practical, the latter especially with reference to dosage, toxicity and unexpected actions in medication of persons at high altitudes. Macht[2] has recently claimed that digitalis is more toxic at high altitudes in the Blue Ridge Mountains, Rocky Mountains, and the Tyrolean Alps than at Baltimore. The continued studies of digitalis along different lines in this laboratory prompted us to include determinations at high altitudes of the emetic and fatal doses in pigeons and of fatal doses in cats. The results obtained are generally consistent with Macht's

[1] Loewy, A., *Physiologie des Höhenklimas*, 1932, 414, Springer, Berlin.

[2] Macht, *Am. J. Physiol.* (Proc.), 1931, **97**, 540.

claims, and indicate the desirability of similar investigations with other drugs.

The plan of our experiments was as follows: A potent tincture of digitalis which has been assayed for several years by the pigeon method[3] was used. The pigeon-emesis method was used to great advantage, since it was possible to repeat the test on the same pigeons, first at one altitude and then at the other. Thus, any changes were due solely to altitude, exclusive of individual variations, which offer greater difficulties in fatal-dose methods. However, the fatal doses for pigeons were also determined, thus providing a check on the results with emetic doses.

Actually, 2 large groups of pigeons were used. One group was used for determining the emetic doses, and their distribution, at San Francisco. A week later, *i. e.*, after recovery, the same was done at Tioga Pass in the Sierra Nevada Mountains. After keeping these pigeons another week at Tioga, the fatal dose was determined. Using another group of fresh pigeons, the emetic doses were first determined at Tioga and a week later at San Francisco; after another week at San Francisco, the fatal dose was determined. The usual procedure for determining the intravenous fatal dose in cats was used, the cats being anesthetized with barbital intraperitoneally. Two groups of 8 cats each were injected, one at San Francisco and the other at Tioga. The estimated altitudes and barometric pressures were: San Francisco, 0 and 760 mm.; Tioga Pass, 10,000 ft. (3000 + M.), and 526 mm., a decrease of 234 mm.

The results with the 2 corresponding groups of pigeons were put together in Table I as they were practically the same.

TABLE I.
Emetic and Fatal Doses of Digitalis in Pigeons at Different Altitudes.

	Digitalis: mg. per kilo body weight													
Elevation	5	10	15	20	25	30	60	90	98	105	120	135	143	150
	% Emesis*									% Mortality†				
San Francisco (0)	0	0	29	50	*54*	75	0	0		0	40	*57*‡	80	100
Tioga Pass (10,000 ft.)	0	25	*63*	67	75	96	0	20	40	*67*§	80			100

*24 pigeons for each dose; †at least 5 pigeons for each dose; ‡12 pigeons; §7 pigeons.

Emetic Doses in Pigeons. A total of 288 tests with 6 doses of digitalis, ranging from 5 to 30 mg. per kilo body weight, were made in 144 different pigeons. The results in the table show conclusively that all doses, except the 5-mg. dose which was ineffective at both

[3] Hanzlik, *J. Pharm. Exp. Therap.*, 1929, **35**, 363.

altitudes, caused a greater number of pigeons to vomit at Tioga than at San Francisco; actually, from 17 to 34%, median 21%, more vomited at Tioga. The M. Em. D. was 25 mg. per kilo at San Francisco, and 15 mg., or about 40% less, at Tioga. If compared with the dose of 20 mg. per kilo which caused 50% of pigeons to vomit at sea level, the M. Em. D. at Tioga was still 25% less. Nearly all the pigeons vomited after 30 mg. per kilo of digitalis at Tioga, but only 75% at San Francisco. Thus, a greater potency of digitalis at the high altitude was demonstrated by data on the distribution of emetic doses and the minimum emetic dose. In general, a 1% increase in number of pigeons vomited for each fall of 11.6 mm. barometric pressure, or rise of 418 feet in altitude; somewhat similar was the variation in emetic potency. It follows that for an equal degree of pharmacological activity at sea-level, the dosage of digitalis at an altitude of 10,000 feet should be reduced from 1/4 to 2/5.

Fatal Doses in Pigeons. The fatal doses of digitalis were determined in 74 pigeons. The results essentially confirmed those with emetic doses. The mortality was greater for each dose tried at Tioga, except the dose of 60 mg. per kilo, which failed to kill at both altitudes. The surely fatal dose at both altitudes was 150 mg. per kilo. The mortality was about 40% (median) greater at Tioga for comparable doses, the differences being even more striking with doses of 98 and 105 mg. per kilo. The M. F. D. at San Francisco was 135 mg. per kilo, and 105 mg., or about 22.2% less, at Tioga. The mortality was increased 1% for each 7 mm. fall of barometric pressure, or 252 feet rise in altitude. It follows that for greater safety in medication with digitalis at high altitudes the dosage should be definitely less than at sea-level.

Fatal Doses in Cats. The 16 cats used gave the following fatal doses of digitalis in mg. per kilo body weight: at San Francisco, median 108, range 87 to 190, and at Tioga, median 127, range 57 to 170. Accordingly, the extreme doses at the high altitude were less than those at sea-level in agreement with the tendency in pigeons. However, the median doses showed the reverse tendency, due probably to the greater variations in the cats and the small number used than was the case with pigeons. A comparable number of cat-tests was obviously out of the question, whereas the pigeon-tests were made on a large and significant scale with the greatest ease and convenience, which illustrates some decidedly practical advantages in the pigeon method. For the present, the results of the cat-tests

must be regarded as less conclusive than those of the pigeon-tests. Macht's results were obtained with cats, but he gives no data.

Conclusions. The emetic and fatal doses of digitalis in significant numbers of pigeons were found to be 40 and 22% less, respectively, at an altitude of 10,000 feet than at sea-level. A similar tendency was shown by the extremes in fatal doses for cats, but the results were inconclusive, due probably to greater variations in cats and smaller numbers used. The higher potency of digitalis at high altitudes reflects changes in state of the emetic and circulatory functions at high levels and indicates the desirability of reducing the dosage of the drug at high levels so as to avoid undesirable and toxic reactions.

6397

Artificial and Hereditary Suppression of Sacral Vertebrae in the Fowl.*

C. H. DANFORTH.

From the Department of Anatomy, Stanford University.

An understanding of the action of hereditary factors in regulating structural expression is likely to be facilitated by finding ways of reproducing the same effects by other than genetic means. To this end, the conditioning of either of 2 allelomorphic forms irrespective of the genotype is a principal desideratum. The present note records the few fragmentary data obtained from an attempt in this direction. Suppression of several of the sacral and caudal vertebrae in the fowl producing the condition known as "rumplessness" has long been known, and has been studied by Du Toit,[1] Dunn,[2] and Landauer,[3] who have described the anatomy and genetics of the condition. The latter authors have also pointed out the occurrence of occasional spontaneous non-hereditary cases which differ little, if at all, from those of the hereditary type. Owing to the extreme rarity of the sporadic cases (not over 1 in 1000, according to Dunn), it has hitherto seemed improbable that much could be learned of their genesis.

* Aided in part by a grant from the Rockefeller Fluid Research Fund of Stanford University.

1 Du Toit, P. J., *Jenaische Z. f. Naturwissensch.*, 1913, 49.

2 Dunn, L. C., *J. Hered.*, 1925, **16**.

3 Landauer, Walter, *J. Hered.*, 1928, **19**.

The simultaneous appearance of 3 such cases among a small number of chicks hatched for another experiment suggested a search for the cause of the anomaly. Since the abnormal chicks were from artificially incubated eggs, the occurrence of some disturbance before or at the time of most rapid differentiation of the caudal end of the embryo seemed probable. Consequently tests were made in which the temperature was varied in different ways during the first 6 or 7 days of incubation. In one series it was possible through the kindness of Dr. Landauer to test eggs from fowls which were hereditarily rumpless, and should consequently be expected to produce a considerable proportion of rumpless chicks (Dunn and Landauer). From 116 eggs of the rumpless strain there were obtained 24 chicks which reached a stage where their condition could be determined with reasonable certainty. From 84 eggs of a normal (White Leghorn) strain 52 usable chicks were secured. The results were as follows:

Eggs from the rumpless strain incubated at 97°-98° throughout gave 6 normal and 1 doubtful embryo; 97°-98° for 4-6 days followed by 103° gave 5 normal and 5 rumpless; 103° throughout gave 4 normal and 3 rumpless.

Eggs from the normal strain incubated at 97°-99° throughout gave 21 normal; 97°-99° for 5-6 days followed by 2 or more at 103° gave 11 normal; 97°-99° for 6 days, 103° one day, then 97°-98° gave 1 normal, 1 rumpless; 99°-100° for 5 days, 101° one day, then 103° gave 2 normal, 1 rumpless; 103° throughout gave 7 normal; 103° for 4 days, then 97°-99° gave 5 normal, 2 rumpless; 104°-105° for 4 days followed by 101°-103° gave 1 normal.

Of the 52 chicks in this series from normal parents there were 4, or about 7½%, that were rumpless. All of these came from eggs which had been subjected to fluctuating temperatures during the first week of incubation. There is little indication that a consistently low temperature even 4° to 6° below normal, is in itself conducive to rumplessness.

Owing to lack of material it has not been possible to experiment further with eggs from rumpless strains, but similar tests with eggs from other normal strain have continued to yield about the same proportion of rumpless chicks. Some of the latter embryos have been sectioned and compared with genetically rumpless specimens which they are found to resemble in general features and in showing a considerable range of variation. The conclusion consequently seems justifiable that by this admittedly crude and non-quantitative method some advance has been made toward solving one part of the prob-

lem. A certain percentage of non-hereditary rumpless specimens can be produced.

Presumably the condition is brought about through a slowing of the growth rate just when the posterior end of the body should be in the state of its most rapid differentiation, a time, according to Stockard,[4] when any arrest is likely to produce lasting effects. That the condition is one of arrested development is further indicated by a rather frequent occurrence of ectopic viscera among these specimens.

These results with eggs of normal stock suggest that the genetic form of the trait may similarly be determined by a differential retardation at some critical moment. The available eggs were not sufficient to test this point, but so far as they go the data are suggestive, inasmuch as from eggs incubated at consistently low temperatures there were 6 normal embryos and one doubtful, while from those incubated wholly or in part at higher temperatures there were 9 normal and 8 rumpless chicks. It is regretted that it has not yet been possible to make tests to determine whether genetically rumpless embryos may have their general metabolism sufficiently slowed during the first week of incubation to reduce the differential to a point that would permit development of normal sacral and caudal vertebrae.

At present something can be done toward making an embryo of normal genotype develop, or fail to develop, a tail. It remains to be determined how a similar control may be exercised over a genetically rumpless embryo.

6398

Effect of Intravenous Injection of Ethyl Alcohol on Gastric Secretion in Man.*

H. W. NEWMAN and H. G. MEHRTENS.

From the Division of Neuropsychiatry, Stanford University Medical School.

Of the tests of gastric secretion, that employing alcohol as the stimulus is one more commonly used clinically. The recent use of

[4] Stockard, Charles R., *Am. J. Anat.*, 1921, **28**.

* Supported by a grant from the Rockefeller Fluid Research Fund of Stanford University Medical School.

alcohol by vein as a surgical anesthetic[1, 2] suggested the question as to whether intravenous alcohol would prove as active a stimulus to gastric secretion as that administered by mouth.

In the alcohol test meal as used in practice, 50 cc. of 7% alcohol is introduced into the previously emptied stomach, and the contents aspirated at 15-minute intervals for titration.[3] We have attempted a comparison of the changes in gastric acidity, blood alcohol, and gastric juice alcohol brought about by this procedure with those produced by varying doses of alcohol intravenously. In all cases the solution used in the intravenous test meal was 25% ethyl alcohol in normal saline, injected at a constant rate of 10 cc. per minute. The alcohol determinations were made by the method described by Cannan and Sulzer.[4]

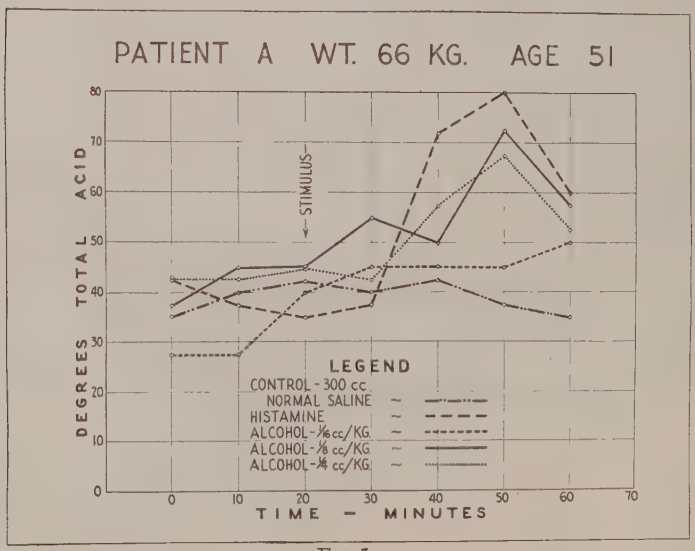


FIG. 1.

Figure 1 shows that in this patient the injection of 300 cc. of normal saline at the prescribed rate as a control had no appreciable effect on gastric acidity, as determined by titration of samples aspirated continuously over successive 10-minute periods. It also shows the response of this same patient to doses of 1/16, 1/8, and 1/4 cc.

[1] Garcia, Miguel, 1929, *E. Mesones. Imp.*, Mexico.

[2] Fohl, Th., *Arch. f. Klin. Chir.*, 1931, **165**, 641.

[3] Cheney, W. F., *Oxford Monographs*, 1928.

[4] Cannan, R. K., and Sulzer, R., *Heart*, 1924, **11**, 141.

of 95% alcohol per kg. body weight intravenously as the 25% solution, and also to 0.01 mg. histamine per kg. subcutaneously. It will be noted that 1/8 cc. of alcohol per kg. gives a maximal response, but that the response is not so great as that to histamine.

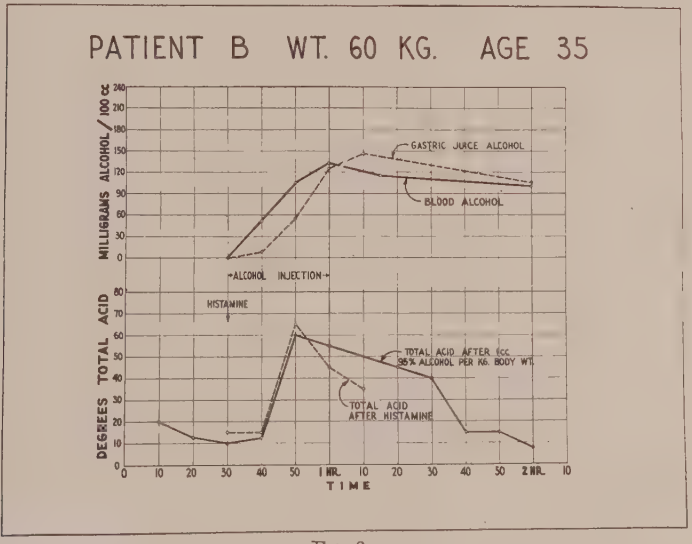


FIG. 2.

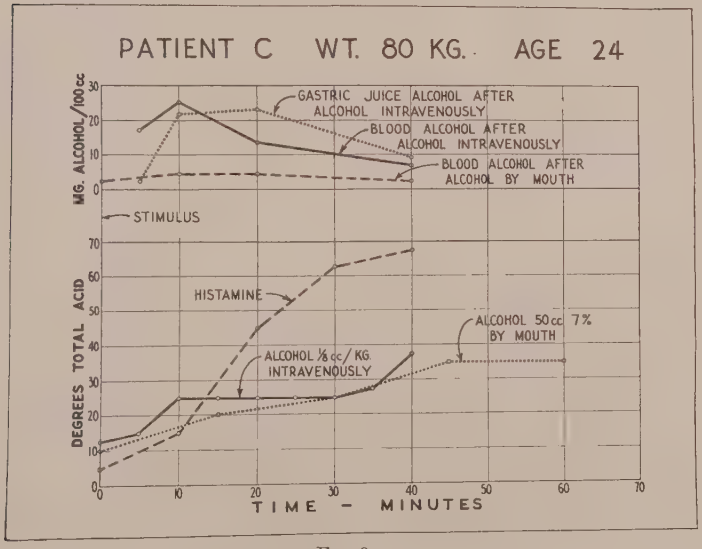


FIG. 3.

Figure 2 shows the response of another patient to 1 cc. of 95% alcohol per kg. intravenously as the 25% solution. Here again the response does not equal that to histamine. The alcohol content of the gastric juice closely follows that of the blood.

Figure 3 shows the response of a third patient to the oral alcohol test meal, to 1/8 cc. of 95% alcohol intravenously, and to histamine; as well as the blood alcohol curve during the oral and the intravenous meal. There is no significant difference evident in the responses to alcohol by the 2 routes, indicating that the mechanism in both cases may be the same. Here the response to histamine far exceeds that to alcohol orally or intravenously.

The foregoing work merely indicates the field opened up by this new method of approach. We will report an investigation of psychological and psychiatric problems by this method in future papers.

Conclusions. 1. Alcohol intravenously in man produces an increase in gastric acidity. 2. 1/8 cc. of 95% alcohol per kg. body weight intravenously as a 25% solution in normal saline is as effective a stimulant of gastric secretion as is the standard 50 cc. of 7% alcohol by mouth. 3. The response to alcohol intravenously up to 1 cc. per kg. is not as great as that to histamine. 4. The alcohol content of the gastric juice closely follows that of the blood. 5. The blood alcohol during the oral alcohol test meal is much less than after the least effective dose by vein. 6. The gastric juice alcohol after the intravenous injection is minute compared to the 7% administered by mouth. 7. From 5 and 6 above, it would seem possible that the seat of action of alcohol as a stimulus to gastric secretion lies neither in the general circulation, nor at the surface of the gastric mucosa, but somewhere between the two. Further investigation of this point is in progress.

6399

Induction of Ovarian Growth with an Extract Made From Blood of Pregnant Women.*

C. F. FLUHMANN.

From the Department of Obstetrics and Gynecology, Stanford University School of Medicine.

In a previous communication[1] a method was described for preparing a crude but highly active extract of an ovary-stimulating principle from blood of pregnant women. It was also pointed out that this extract readily induced luteinization in the ovaries of immature rats, but as had already been observed with the "anterior-pituitary-like" principle from the placenta (Collip, *et al.*[2]) and with prolan (Evans, Meyer, and Simpson[3]), it does not stimulate the rapid development of graafian follicles such as results from the implantation of fresh anterior pituitary tissue. In 5-day experiments it has thus not been possible to produce any increase in ovarian weight beyond the limit found in normal adult animals.

It has now been found, however, that marked ovarian growth may be induced by using larger doses over longer periods of time. A total of 50 animals were given 2 daily injections of the extract in doses equivalent to 0.22 ccm. of the original blood plasma and the administration continued for periods up to 29 days. The treatments were begun in rats 21 or 22 days of age. In a group of 4 rats injected for 3 weeks the average body weight was 58 gm. and the weights of the ovaries varied from 126 to 174 mg., while after 4 weeks the body weight of 6 rats averaged 94 gm. and ovarian weights of from 84 to 293 mg. were obtained. The average weight of the 2 ovaries in a series of 13 uninjected control rats, 49 days of age, body weight 79 gm., was 19 mg. The average ovarian weights of 9 of these which had not reached sexual maturity was 15 mg., while it was 30 mg. in 4 whose vaginal orifice had become established.

These results would seem to show that a marked increase of ovarian weight may be produced in immature rats by adequate dosage

* Supported by a grant from the Rockefeller Fluid Research Fund of Stanford University School of Medicine.

1 Fluhmann, C. F., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1193.

2 Collip, J. B., Thomson, D. L., McPhail, M. K., and Williamson, J. E., *Can. Med. Assn. J.*, 1931, **24**, 201.

3 Evans, H. M., Meyer, K., and Simpson, M. E., *Am. J. Physiol.*, 1932, **100**, 141.

and the prolonged administration of an ovary-stimulating extract made from the blood of pregnant women. The "time factor" in this phenomenon is thus an important feature in comparing the effects of this hormone with those of fresh anterior hypophysial implants.

6400

Lipolytic Activity of Rat Tissues in Experimental Leprosy.*

GEORGE EMERSON, H. H. ANDERSON AND C. D. LEAKE.

From the Pharmacological Laboratory, University of California Medical School, San Francisco.

Walker and Sweeney[1] suggested that an indirect or physiological method of therapeutic action of chaulmoogra oil in leprosy might be stimulation of non-specific lipolytic activity of the tissues which would attack the fatty capsule of acid-fast bacilli. We have undertaken the study of the lipolytic activity of leprous tissue in experimental rat leprosy in comparison with that of normal tissues of the same animal, and the determination of whether or not the observed ratios may be altered by treatment. We decided to ascertain first, however, what effect the presence of leprosy in the body might have on the tissue distribution of lipase in comparison with that noted in non-infected normal animals. Our observations on this point are reported herewith.

Estimations of the lipolytic activity of representative tissues were made by Loevenhart's method[2] on 8 healthy adult female rats and on 22 female leprous rats. The latter had been inoculated with a buffered saline suspension of finely ground rat leprous tissue from 140 to 194 days previously by Dr. E. L. Walker and Miss M. A. Sweeney. The source of this material was spontaneous leprosy in wild rats obtained from the U. S. P. H. Plague Laboratory in San Francisco, transferred about every 6 months through the Burlingame strain of albino rats for 10 generations without apparent al-

* Part of a cooperative study of the chemotherapy of leprosy conducted by the Hooper Foundation for Medical Research and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by the Christine Breon Fund.

[1] Walker, E. L., and Sweeney, M. A., *J. Inf. Dis.*, 1920, **26**, 238.

[2] Kastle, J. H., and Loevenhart, A. S., *Am. Chem. J.*, 1900, **24**, 491; Loevenhart, A. S., *Am. J. Physiol.*, 1902, **6**, 331.

teration in virulence. Definitely palpable lesions were present at the time of death. The 8 healthy rats and half the leprous rats were killed by a blow on the head while the remainder of the leprous rats died from pneumonia. Tissues were taken at death in all cases, and particular care was used to grind them thoroughly. Estimations on individual tissues were made in triplicate and were in agreement within the limits of error of titration with a micro-burette, that is, 0.005 cc. This gave an approximate error, using N/20 KOH, of ±0.005 in the case of the percentage hydrolysis figures for liver, and of ±0.05 in those for leprous tissue.

TABLE I.

Lipolytic Activity of Various Tissues in Leprous and Normal Rats as Estimated by Percentage Hydrolysis of Ethyl-butyrate.

Tissue	Leprous Rats				Normal Rats	
	Early stage small lesion		Late stage large lesion			
	No. animals	% hydrolysis	No. animals	% hydrolysis	No. animals	% hydrolysis
Lepromatous	11	0.17±0.02	11	0.14±0.01	—	——
Subcutaneous	8	0.83±0.07	2	1.24±0.19	4	0.99±0.28
Abdominal Muscle	9	0.35±0.14	4	0.41±0.13	5	0.57±0.08
Gluteal Muscle	9	0.31±0.09	8	0.25±0.09	8	0.42±0.19
Liver	11	3.11±0.98	10	1.42±0.41	8	3.15±0.78
Lung	9	1.67±0.78	10	0.53±0.14	6	1.40±0.53
Heart	8	0.34±0.07	6	0.27±0.06	6	0.60±0.10
Kidney	8	3.00±0.87	8	2.67±0.35	5	3.03±0.47
Spleen	9	0.35±0.13	10	0.45±0.34	7	0.26±0.06

A summary of our findings is given in the table. Since the pathological status of the leprous lesion is not wholly dependent on the time after inoculation, we decided arbitrarily to use the degree of local involvement at the site of infection as a criterion of "early" (mild) or "late" (severe) leprosy. An early lesion is small, may be localized or diffuse, but is uneven in consistency with no skin changes at the site and no slough. A late lesion is large, compact, firm, with degenerative skin changes and frequent sloughing. The estimations of the lipolytic activity of the various tissues are expressed in percentage hydrolysis of the ethyl-butyrate substrate after 30 minutes at a temperature of 38°C.

Our observations indicate 3 outstanding facts regarding the relation between experimental leprosy and the lipase content of tissues in infected rats: (1) leprous tissue is significantly lower in lipolytic activity than other tissues in infected or normal rats; (2) leprous tissue from different animals is remarkably constant in lipolytic action in comparison with other tissues in infected or normal rats, and (3) the presence of "late stage large lesion" leprosy significantly

lowers the lipolytic activity of most tissues in the body, with the probable exception of the spleen, in comparison with tissues from non-infected normal rats. Even in "early stage small lesion" leprosy there seems to be a tendency in some tissues of the infected rats, especially heart and abdominal muscle, to be significantly lower in lipolytic action than comparable tissues from healthy non-infected rats.

6401

Renal Pigmentation Following Ingestion of Psyllium Seed.

EATON M. MACKAY, ERNEST M. HALL AND FRANCIS M. SMITH.

From the Scripps Metabolic Clinic, La Jolla, California, and the Department of Pathology, University of Southern California School of Medicine, Los Angeles.

Psyllium seed, often known as "flea seed", is the seed of a plant, *plantago psyllium,* which grows in southern European countries. This seed finds current vogue as a laxative. The seeds have the property of swelling and becoming gelatinous on contact with moisture. Their laxative action has generally been attributed to this physical change and explained on a purely physical basis.[1] During an experimental study of the effect of the roughage in the diet on the intestinal tract of the albino rat psyllium seed was used as one source of a residue substance. On sacrificing these animals after 125 days on the diet it was found that the kidneys were black in color.

Sagittal section of these organs showed most of the pigment material in the cortex with a still blacker area of demarkation toward the medulla (Figs. 1A and 1B). Microscopic examination revealed many coarse to moderately fine brown granules in the epithelial cells of the tubules (Fig. 2). These granules were most abundant in the proximal convoluted tubules and in the loops of Henle. They tended to accumulate in the basal portions of the cells and about the nuclei. Only an occasional granule was found in the lumen. The pigment was not uniformly spread through the cortex but tended to accumulate more abundantly in groups of 6-12 or more tubules. Considerable pigment was found in the outer portions of Henle's loop but none was seen in the deeper parts of the medulla. The pigment failed to give the reaction for iron when sections were treated

[1] Macht, D. I., and Black, J. A., *Proc. Am. Physiol. Soc., Am. J. Physiol., Soc., Am. J. Physiol.,* 1932, **101**, 71.

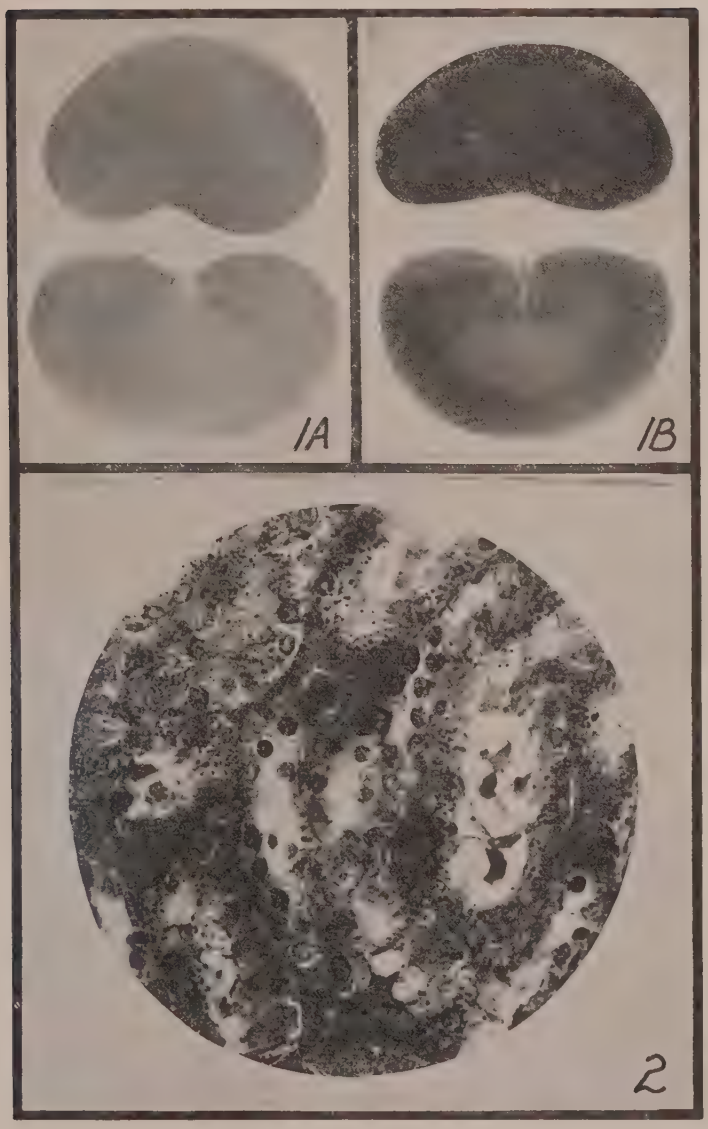


PLATE 1.

Fig. 1A—Kidneys of rat fed the control diet for 125 days.

Fig. 1B—Kidneys of rat fed a diet containing 25% ground psyllium seed for 125 days.

Fig. 2—Microphotograph (high power) showing pigment in the convoluted tubules of kidney pictured in figure 1B.

with potassium ferro-cyanide and hydrochloric acid, neither did it stain with basic fuchsin. The kidneys of the control rats to which no psyllium seed was given were entirely free of pigment.

The rats in which the black kidneys were first found received a mixture containing 75% of a control diet[2] and 25% of finely ground psyllium seed. It was impossible to feed whole seed to the rats for they simply ate the food around the seeds and discarded the seeds. Since we desired to know whether human kidneys might ever become pigmented as a result of the therapeutic use of the whole seed an experiment was carried out with a litter of 6 young dogs. Two received a diet of commercial canned dog food. Two more received the same food mixed with 25% of whole psyllium seed and the third pair the same except that the seed had been ground. After 30 days on these diets only the dogs which had been fed the ground seed had gray black kidneys when they were killed. The pigmentation was grossly not as marked as in the rats. No pigment granules were found on microscopic examination in the kidneys of these dogs or in any of the others. These animals had been fed psyllium seed for only 30 days in contrast with the rats which showed microscopic as well as gross pigmentation, which had been on the diet for 125 days. In 2 rats fed psyllium seed in the diet for 10 days, although the kidneys were grossly darker than the controls, no pigment was found on microscopic examination. The livers were also somewhat dark in appearance but failed to show pigment on microscopic examination. It might be noted that for their size the psyllium seed fed rats always had heavier kidneys than their controls. It is not certain, however, that they were swollen, for the rats were undernourished and the kidneys may simply have been of normal size.

It seemed probable that the renal pigmentation was due to the black pericarp of the seeds but when the bulk of the mucilaginous material was dissolved out of crushed seeds with hot water and the dried shells fed to rats in the usual diet at a concentration of 30% no pigmentation of the renal organs followed. Flaxseed, which has physical characteristics very similar to psyllium seed failed to produce any pigmentation of the kidneys when fed in the same concentration, either whole or ground.

We obtained no evidence as to the chemical nature of the coloring substance in the kidneys. Strong alkali extracted a dark brown substance from the seeds but not from pigmented kidneys. The color in the kidneys faded slowly when they were preserved in neu-

[2] Addis, T., MacKay, E. M., and MacKay, L. L., *J. Biol. Chem.*, 1926, **71**, 139.

tral or acid formalin or ethyl alcohol and more slowly in chromate fixing solutions.

Conclusions. The addition of a large quantity of ground psyllium seed to the diet of albino rats or dogs is followed by a darkening of the kidneys when examined grossly. If the feeding is continued for a longer period brown pigment granules become evident microscopically in the renal tubules. Whole psyllium seed produces no renal pigmentation.

6402

Method for Determining Shape of Colloidal Particles; Application in Study of Tobacco Mosaic Virus.

WILLIAM N. TAKAHASHI AND T. E. RAWLINS.
(Introduced by T. D. Beckwith.)

From the Division of Plant Pathology, University of California, Berkeley, Calif.

According to Freundlich,[1] when a sol containing rods, discs, or leaf-shaped colloidal particles is flowing through a tube the particles become oriented with the longest axis of the particles parallel to the direction of flow. Discs or leaf-shaped particles near the walls of the tubes also tend to be oriented with their faces parallel to the adjacent wall. Ambronn and Frey[2] reported that sols containing rod-shaped particles are doubly refractive when the particles are oriented by streaming and the direction of observation is perpendicular to the direction of flow. Sols containing discs or leaf-shaped particles show double refraction when the longest axis of the particles is parallel to the direction of flow and the faces of the particles are parallel to the direction of observation.

The above phenomena led us to assume that if a sol containing rod-shaped particles were forced from a small glass tube of circular cross section into the same sol contained in a beaker the orientation of the particles should be the same throughout the stream and all parts of the stream should, therefore, show double refraction. If the direction of flow were reversed and the sol were sucked from the beaker through the small glass tube the sol in the beaker should flow radially toward the mouth of the tube as a center and the long

[1] Freundlich, Colloid and Capillary Chemistry, 1922, E. P. Dutton & Co., N. Y.

[2] Ambronn and Frey, *Das Polarisationsmikroskop*, 1926, Akademische Verlagsgesellschaft M. B. H. Leipzig.

axis of the particles should be oriented parallel to the directions of flow. All regions of the sol in the beaker which are flowing toward the mouth of the tube would be expected to show double refraction except those regions which are flowing parallel to the vibration directions of the crossed nicols. A dark cross should, therefore, be observed in the doubly refractive sol which is flowing toward the mouth of the tube. If a sol containing discs or leaf-shaped particles were forced from the small glass tube the long axis of the particles should be parallel to the direction of flow but only the particles near the lateral walls of the tube would have their faces parallel to the direction of observation. Therefore double refraction would be expected only near the edges of the stream. If the direction of flow were reversed, one should expect that neither double refraction nor a dark cross would be detected in the sol flowing toward the mouth of the tube, since the faces of the particles would not be oriented relative to the direction of observation.

In order to test these hypotheses, sols whose particle shape had been determined by Freundlich[2] and his associates by the application of the Tyndall method, were forced from a small tube (inside diameter 0.5 mm.) into a beaker and were then sucked from the beaker back through the tube. It was observed that vanadium pentoxide, aniline blue, and benzopurpurin sols showed double refraction throughout the stream when forced from the tube and also exhibited double refraction and the dark cross when sucked toward the mouth of the tube, thus showing the behavior which would be expected of sols containing rod-shaped particles.

On the other hand, a ferric oxide sol was found to show double refraction only along the edges of the stream when forced from the tube. Neither double refraction nor a dark cross were observed when the sol was sucked toward the mouth of the tube. As stated above, this is the behavior which would be expected of sols containing discs or leaf-shaped particles.

Freundlich[2] reported that studies involving the application of the Tyndall phenomenon have indicated that vanadium pentoxide, aniline blue, and benzopurpurin sols contain rod-shaped particles and that ferric oxide sols contain discs or leaf-shaped particles. Since the principles involved in the Tyndall method are different from those involved in the stream double refraction method, and since the two methods have led to the same conclusions regarding the shape of the colloidal particles which were studied, it appears that the latter method is probably reliable for determining the shape of particles in sols which exhibit stream double refraction.

Up to the present time no evidence has been available regarding the shape of the particles of the various filterable viruses. We have attempted to determine the shape of tobacco mosaic virus particles by the use of the stream double refraction method and have found that suspensions of the virus showed double refraction throughout the stream when forced from a small tube and exhibited double refraction and the dark cross when sucked toward the mouth of the tube. Juice from healthy plants exhibited no double refraction when forced from the tube or when sucked toward the tube. This experiment was repeated a number of times with juice from different portions of tobacco plants and with tomato plants infected with the same virus. In all cases the juice from infected tissues showed the same type of stream double refraction and the juice from uninfected tissues failed to show detectable double refraction.

The evidence therefore indicates that the virus of tobacco mosaic, or some substance regularly associated with it, is probably composed of rod-shaped particles.

Iowa Section.

State University of Iowa, October 20, 1932.

6403

Control of the Sex Characters in the English Sparrow, Passer domesticus (Linnaeus)*

WARREN N. KECK. (Introduced by Emil Witschi.)

From the Laboratory of the Department of Zoology, University of Iowa.

I. The Male. A series of experiments was carried out to determine the nature of the control of the sex characters, particularly plumage, of the English Sparrow. Previous studies have established that the secondary sexual characters, including plumage, of various breeds of domestic fowl, ducks and pheasants are under the influence of the gonads.

In the first experiment 52 male birds (35 adult and 17 immature) were castrated. At the time of the operation feathers were plucked from areas showing sexual dimorphism. The regenerated feathers were always of the male type. The capon assumes the normal male plumage and in this respect is similar to domestic fowl.

The bill of the adult male during the breeding season is blue-black in color but during late August and early September the bill becomes a light horn color. This light color is retained during the winter but with the approach of the breeding season the bill again takes on the black color. When a male with a black bill is castrated the color changes to a light shade, characteristic of the winter condition when the gonads are inactive. The color change becomes noticeable at the base of the mandible 10-14 days after the operation. Three weeks later the bill has completely changed color.

In the second series daily intramuscular injections of female sex hormone (prepared from the urine of pregnant women) were given 12 capons. The dosage varied from ½ to 10 rat units per day for

* The expenses of this investigation were supported in part by the Committee for Research in Problems of Sex of the National Research Council; grant administered by Dr. Emil Witschi.

a period of 30 days, a given capon receiving the same dose each day. Feathers were plucked at the time the injections were started. The regenerated feathers were of the normal male type. Experiments using Brown Leghorn and Sebright Bantam capons demonstrated that 1-2½ rat units of female sex hormone per 100 gm. of body weight given daily for a period of 30 days influenced regenerating feathers toward femaleness. Some of the sparrow capons received as much as 40 rat units per 100 gm. body weight of the same extract used on the Leghorns and Bantams but with no apparent effect on the plumage.

II. The Female. Forty-eight female sparrows were ovariotomized. Of this number 30 were adult and 18 immature. At the time of the operation feathers were plucked from sexual dimorphic areas. The regenerated feathers were invariably of the normal female type. In no case was there any indication of an influence toward male feathering that is so characteristic of poulards of fowl and ducks. Post-mortem examination revealed no macroscopic rudiments or regeneration on the site of the left ovary. Neither was there any compensatory hypertrophy of the right rudiment. In all cases the oviduct was small and immature in appearance. Histological examination of 6 poulards proved the operation to be complete.

Subcutaneous and intra-abdominal testis implants were made on 15 poulards. Post-mortem examination of 3 of these birds revealed active implants. Sexual dimorphic feather areas have been deplumed twice and each time they have regenerated normal female feathers. Some of the individuals are still under observation but to date have failed to show any modifications toward male feathering.

Six immature female birds were daily injected with 2 rat units of female sex hormone for a period of 9 days. The oviducts were then dissected out and weighed. They showed hypertrophy as compared with the oviducts of controls in that they were more convoluted, swollen and with a richer vascular supply. The oviducts of the test individuals averaged 33⅓% heavier than those of controls.

From the above results it appears that the secondary sexual characters of the English Sparrow can be divided into 2 categories. (1) Those under the influence of secretions from the gonads: namely, the bill coloring of the male; and the oviduct of the female.[1] (2) Those under a genetical control and not influenced by gonad hormones, namely, male and female plumages.

[1] Juhn, Mary, and Gustavson, R. G., *J. Exp. Zool.*, 1930, **56**, 31.

6404

Relationship Between Permeability to Bromides and Protein Content of the Cerebrospinal Fluid.

WM. MALAMUD, W. R. MILLER AND B. M. MULLINS.

From the Psychopathic Hospital, State University of Iowa.

In previous communications[1, 2, 3, 4] we reported the results of investigations of the passage of bromides from the blood into the cerebrospinal fluid in different types of mental diseases. The determination was carried out by the macrocolorimetric method devised by Walter.[1] In subsequent investigations a microcolorimetric modification of this method[5] was used with similar results. To determine the mechanisms responsible for the peculiar distribution of the bromides between the blood and cerebrospinal fluid in normals and its modifications in mental diseases, it was deemed necessary to make determinations of other cerebrospinal fluid contents simultaneously.[6, 7] For variations in these other substances may influence the distribution of the bromides.

In this communication we report the relationship of total protein content in the cerebrospinal fluid and the bromide distribution ratio. In 356 consecutive admissions to this hospital, representing most of the mental diseases met with in hospitals for the insane, the total protein content of the cerebrospinal fluid was determined by the Ayer method,[8] at the same time determinations were made of the bromide distribution ratio.

The results were as follows: There was a tendency for the high protein contents to be massed with the high bromide contents and the low protein with the low bromide. This was not consistent, however, as in some cases with high protein a decreased bromide

[1] Malamud, Wm., Fuchs, D. M., and Malamud, N., *Arch. Neurol. and Psychiat.*, 1928, **20**, 780.

[2] Malamud, Wm., Wilson, R. B., *Arch. Neurol. and Psychiat.*, 1929, **22**, 1135.

[3] Malamud, Wm., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 477.

[4] Malamud, Wm., Rothschild, D., *Arch. Neurol. and Psychiat.*, 1930, **24**, 348.

[5] Malamud, Wm., and Mullins, B. M., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 871.

[6] Malamud, Wm., Hayward, E. P., *Z. ges. Neurol. und Psychiat.*, 1930, **128**, 295.

[7] Rothschild, D., and Malamud, Wm., *Arch. Neurol. and Psychiat.*, 1931, **26**, 829.

[8] Ayer, J. B., Dailey, M. E., Fremont-Smith, F., *Arch. Neurol. and Psychiat.*, 1931, **26**, 1038.

content was found and vice versa. The coefficient of correlation in this group was 0.5734 with a probable error of ±0.0280.

It was somewhat different, however, in the case of the determinations in the special syndromes. (1) The group of cases diagnosed psychopathic personality (37) showed a coefficient of 0.7769 with a probable error of ±0.0440. (2) The group of schizophrenias (82) showed a coefficient of correlation of 0.7000 and probable error of ±0.0380. (3) In the cases diagnosed "without psychosis" (33) the coefficient of correlation was 0.6781, the probable error ±0.0634. (4) In the cases where the mental disease was caused by or associated with organic disease (63) the coefficient of correlation was 0.6648, the probable error ±0.0474. (5) in the cases of manic depressive psychosis (64) the coefficient of correlation was 0.4181 with the probable error of ±0.0696. (6) In the psychoneuroses (46) the coefficient of correlation was 0.4158 with a probable error of ±0.0823. All the other cases in the total group belonged to different types of diseases, and the small numbers in each did not permit of statistically valid conclusions.

We believe that the results show primarily that whatever relationship there may exist between the protein content and bromide distribution, it is not that of cause and effect. In all the groups, no matter what the correlation coefficients were, there were cases in which high protein contents occurred in the presence of low bromide permeability and vice versa. We must conclude, therefore, that the peculiar distribution of the bromides cannot be *caused* by the variations in the protein contents of cerebrospinal fluid. The tendency towards correlation, however, would suggest the possibility of similar mechanisms for both in some cases. It is of interest to note that the highest coefficients of correlation were found in the cases of (1) "psychopathic personalities without psychoses" and (2) in the other cases diagnosed "without psychosis" on the one hand, and (3) the organic diseases, as well as (4) the schizophrenias, on the other.

6405

Cost of Work in Relation to Basal Metabolism.

J. T. MC CLINTOCK AND STELLA PAISLEY.

From the Department of Physiology, College of Medicine and Child Welfare Station, University of Iowa.

The extra energy output caused by walking was determined in 65 children, boys and girls, ranging in age from 11 to 14 years. The procedure followed was similar to that of Benedict and Murchhausen.[1] Our results show a general average cost per horizontal kilogram meter for the boys of 0.6449 gm. calories and for the girls 0.5949. The general average for the entire group of boys is approximately 20% higher than the 0.538 gm. cal. given by Smith[2] as an average for 8 normal men examined by him and which he says conforms to the results of Benedict and Murchhausen. The average basal metabolic rate for the group of boys included in our studies was 47.5 cal. per sq. meter surface according to the figures recorded by Du Bois[3] for boys of different ages. This basal rate of 47.5 cal. is also 20% higher than given for adult men in the same table.

Accepting the value of 0.538 gm. cal. reported by Smith[2] as a fair average cost per H. Kgm. M. for horizontal walking by normal adult men and using the Du Bois[3] standard table of B.M.R. for different ages and sex we should be able, if there is any relationship between basal metabolism and unit cost of walking, to calculate the probable cost of a similar activity in each of the different age groups of boys and girls. The calculated values and our experimentally obtained results are set out in Table I for each of the age groups.

TABLE I.

Age	B.M.R.	Girls. Obtained Value gm. cal.	Calculated Value gm. cal.
11	44.5	.6459	.6339
12	43.0	.6045	.6126
13	42.0	.5850	.5982
14	41.0	.5441	.5841
		Boys	
11	48.5	.6527	.6605
12	47.5	.6521	.6469
13	47.0	.6316	.6400
14	46.0	.6434	.6265

[1] Benedict and Murchhausen, Pub. No. 231, Carnegie Institute of Washington.

[2] Smith, Henry M., Pub. No. 309, Carnegie Institute of Washington 143.

[3] Du Bois, Eugene F., Basal Metabolism in Health and Disease, 1924.

While there is not perfect agreement between the obtained and calculated values, there is for this type of work such close accord that we feel justified in drawing the conclusion that the unit cost of work such as walking in normal individuals varies directly with the basal metabolic rate. This finding is in harmony with the results of Plummer and Boothby,[4] from their study of the energy output in horizontal walking by individuals with high basal rate due to hyperthyroidism.

[4] Plummer, H. S., and Boothby, Wm., *Am. J. Phys.*, 1922, **63**, 406.

New York Meeting.

New York Academy of Medicine, November 16, 1932.

6406

Preventing Loss of Weight in the Newborn.

I. NEWTON KUGELMASS.

From the Department of Pediatric Research, Fifth Avenue Hospital, New York.

The newborn is markedly underdeveloped for the duration of human gestation. If birth weights are plotted against gestation times on double-log paper a straight line relationship is found except for a slight deviation in the case of the heaviest animals. The time required for the differentiation of man *in utero* is out of all proportion to all animal species. Yet the product is about one-fourth of the expected birth weight because of the adjustment to the human female. This high degree of underdevelopment makes the newborn's supervision all the more urgent to meet potential pathology with delicate desideratum.

The neonatal growth gradient continues unaltered during the postnatal period. Analysis of the growth of the body and its organs in the fetal period reveals that the course of the growth of all is essentially the same. If the prenatal growth be compared with the postnatal growth by bringing them together on an equivalent scale the characteristic form of growth of the fetal period continues for several months after birth. Therefore loss in weight is exogenous in nature.

Animal species of all sizes, of varied gestation periods, of all scales in evolution receiving no scientific supervision after birth or hatching appear to thrive either immediately or at the utmost after the second day of extrauterine life. Loss of weight in a newborn infant is therefore unnecessary when compared phylogenetically with similar processes in the entire animal kingdom.

Universality of loss of weight in the newborn without exception has glorified the phenomenon into a so-called physiologic law. A century of literature has led early investigators far afield until

recent metabolic studies have indicated that the loss of weight is a result of semi-starvation and dehydration.

The newborn's nutritional requirement can only be fulfilled once it has been alleviated of the symptoms consequent upon the physiologic trauma of birth. The colostrum intake is small and the ingestion of feeding formulas minimal under the most favorable conditions as a result of birth shock. We have therefore been able to prevent the initial loss in weight not by forcing feeding mixtures from the day of birth but rather by the administration of a solution suitable to the physiological needs postnatally. Daily blood studies from birth reveal a hypoglycemia of the newborn not infrequently paralleling a subnormal temperature, a compensated acidosis, a decreased blood volume, an elevated refractive index and serum viscosity and a diminished index of blood clotting function.

The solution, devised for preventing the loss of weight in newborns to the irreducible minimum, was used in 100 cases with an equal number of controls. It consisted of 6% neutral gelatine, 3% dextrose and 0.5% sodium chloride offered every 2 hours throughout the 24 hour cycle. This solution, isotonic and osmolar, has several physiological advantages for the newborn. The gelatine is markedly hydrating, well tolerated and easily assimilable. The sodium chloride favors retention of ingested water by virtue of hydration of the sodium ion as well as by the neutral salt effect. Dextrose raises the level of blood sugar to normal values.

Complemental feeding in 300 control infants merely reduced the loss of weight from the usual 10% anywhere from 6 to 9%. The hydrating solution offered at 2 hour intervals during the first day reduced the loss to 3.4% but when the same solution was offered throughout the 24 hour cycle at the same time intervals the percentage loss in body weight was the irreducible minimum, 1.7%. And these newborns, adequately hydrated, rapidly lost their so-called physiological apathy, somnolence and stupor.

6407

Chemical Differentiation of Nervous and Hormonal Pancreatic Secretion.

T. F. ZUCKER, P. G. NEWBURGER AND B. N. BERG.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

We have previously presented data to show that the secretion of pancreatic juice is continuous[1] and can be clearly demonstrated in dogs if the use of ether anesthesia is avoided. We suggested then that a continuous phase of secretion is produced by means other than secretin. We are now presenting observations concerning the mechanism of secretion and the composition of pancreatic juice produced by various means. Our data confirm the observations of J. Mellanby,[2] who came to conclusions very similar to ours. As new observations we offer those on spontaneous secretion, on the uncomplicated action of pilocarpin, together with more extensive analytical data including comparison with blood.

To obtain juice under conditions of complete exclusion of secretin action, the entire small intestine was removed under amytal or avertin anesthesia after tying off the pylorus. This was accompanied by loss of blood (in the intestine) and considerable shock. In some cases no flow was found, while in others a slow steady secretion was shown. Infusion of glucose solution just before the removal of the intestine materially lessened the shock and tended to restore blood volume. In spite of unfavorable conditions juice could be collected at a rate of 0.01 cc. per minute. This juice was a real pancreatic secretion, *i. e.*, a product specifically of pancreatic cell activity. Its amylase content, the other enzymes running parallel, was as high as 150,000 units per cc., while that for 24 hour samples of mixed juice is maximally 10,000 units and for pure secretin juice maximally 4,000 units. The amylase content was determined by a modified[3] Wohlgemuth method. Because atropin stops the flow of this juice, we were inclined to consider its mechanism as nervous (parasympathetic). To further substantiate such a nervous mechanism we did a series of experiments with pilocarpin.

[1] Zucker, T. F., Newburger, P. G., and Berg, B. N., *Am. J. Physiol.*, 1932, **102**, 193.

[2] Mellanby, J., *J. Physiol.*, 1925, **60**, 85.

[3] Zucker, T. F., Newburger, P. G., and Berg, B. N., *Am. J. Physiol.*, 1932, **102**, 209.

Here we obtained pancreatic secretion in every way similar to that obtained spontaneously under conditions where secretin action was excluded.

In comparing the composition of the various secretions we find: secretin juice: low in enzymes (1,000 to 4,000 amylase units). Low in solids 1-2%. Low in chloride (38 to 77 m. eq.). High in bicarbonate (112 to 140 m. eq.). The total base was uniformly higher than the corresponding blood by 6 to 20 m. eq. ranging from 175 to 192 m. eq. Spontaneous and pilocarpin juice: high in enzymes (70,000 to 150,000 amylase units). High in solids (8-14%). Low in bicarbonate (69 to 77 m. eq.). High in chloride (92 to 101 m. eq.). The total base was approximately that of the blood plasma or lower by about 10 m. eq. (All figures expressed in m. eq. are calculated per kilogram of water. Bicarbonate as an approximation is calculated as difference between total base and chloride.)

These findings have a considerable bearing on pancreatic function. In a certain sense the spontaneous or nervous secretion seems to be functionally of more importance than the secretin juice. It is perfectly evident that pancreatic external secretory activity is not dependent entirely on the gastric activity as was definitely claimed by Bayliss and Starling.[4] Thereby the issues between Pawlow and the British School are acutely revived with strong evidence against the sweeping claims for exclusive hormonal control. A very significant, if not the major part of the enzyme output certainly is due to nervous rather than hormonal action. The experimental animal in marked shock, with its entire small intestine removed, cannot be expected to show more than a fraction of normal nervous secretory activity. It seems remarkable that secretory activity survived at all.

Interpretation of other phases of gastro-intestinal activity is also affected. In achlorhydria, with an absence of the assumed necessary stimulus for pancreatic secretion, a surprisingly normal intestinal digestion has been noted and the high probability of non-hormonal pancreatic secretion suggested.[5] In the absence of gastric HCl the nervous mechanism evidently is sufficient to provide digestive enzymes. The absence of secretin juice affects intestinal digestion principally by a lessened volume of secretion, the loss of bicarbonate in pancreatic secretion being somewhat balanced by the absence of gastric HCl. This latter phase of acid-base balance in the intestine and the amount of water secreted merits further study. We may well look for compensatory phenomena.

[4] Bayliss and Starling, *Erg. d. Physiol.*, 1905, **5**, 665.

[5] Dodds and Bennett, *J. Physiol.*, 1921, **55**, 382.

6408

Bicolor Determination of pH Using Standard Duboscq Colorimeter with Light Filter.

CORNELIUS A. DALY.* (Introduced by A. Knudson.)
With the Assistance of P. James English.

From the Department of Biochemistry, Union University Medical Department, Albany Medical College.

The successful application of light filters in the Folin-Malmros[1] method for sugar suggested the possibility of compensating, in a similar way, the acid color of the bicolor indicators. Employing light filters consisting of the appropriate indicators in an acid solution, a procedure was worked out for determining pH with phenol red, brom cresol purple and brom cresol green.

The acid filter solutions for phenol red and brom cresol purple consists of 0.01% solutions of each indicator made up in acetate buffers having pH values of 5 and 3 respectively. In the case of brom cresol green ethyl alcohol must be added to the acid solution to prevent precipitation of the indicator. A 0.02% solution of the indicator is made up in hydrochloric acid and alcohol so that the final solution is equivalent to 0.1 N hydrochloric acid and contains 35% alcohol.

Battery jars 10 x 10 x 10 cm. or museum jars 16 x 10 x 8 cm. of clear, white glass make satisfactory containers for the filter solutions. The sides of the jars should be painted black, except for a 5 x 8 cm. window to allow the light to pass through. The prepared filter is placed in position between the colorimeter and light, and the instrument "set" in the usual way with the standard in both cups. Readings, which should agree within 0.1 mm. are taken with the left cup set at 1, 5 and 8 mm.

The standard solutions are made by adding 1 cc. of 0.02% phenol red and brom cresol purple, and 0.03% brom cresol green to 20 cc. of the alkaline buffer solutions. The buffers used should have pH values which will completely transform the indicator to the alkaline form. Hastings[2] employs a very dilute solution of sodium hydroxide for this purpose but we prefer buffer solutions. Clark[3]

* George Alexander Research Fellow in Biochemistry, 1930-32, sponsored by The Leopold Schepp Foundation, New York.

[1] Folin, O., and Malmros, H., *J. Biol. Chem.*, 1929, **83**, 115.

[2] Peters, J. J., and Van Slyke, D. D., Quantitative Clinical Chemistry, Methods, Baltimore, 1932, 801.

[3] Clark, W. M., The determination of hydrogen ions, Baltimore, 1928, 94.

gives the pH values required by the different indicators for transformation to the alkaline form.

Add 0.5 cc. of the dilute indicator solution to 10 cc. of the unknown, place in left cup and set at 10 mm. The right cup containing the standard is adjusted until the fields match. The reading of the standard multiplied by 10 gives the per cent alkaline form of the indicator in the unknown. pH values are readily obtained from a dissociation curve of the indicator. The standard solutions should be made up fresh every few determinations since the strong colorimeter light seems to affect the intensity of color.

The method is sensitive, under favorable conditions, to changes of ±0.02 pH. However the absolute accuracy of the procedure depends upon a correct evaluation of pK′ in the solution being investigated. Also it is essential to check the pK′ for each new lot of indicator used. The value obtained is constant over the main pH range of the indicator, which is approximately 1.1 pH units for the dyes studied. A wider range can be utilized if the changing values for pK′ are employed in plotting the extremities of the dissociation curve.

6409

Note on the Susceptibility of Certain Strains of Hemolytic Streptococcus to a Streptococcus Bacteriophage.

REBECCA C. LANCEFIELD. (Introduced by Homer F. Swift.)

From the Hospital of the Rockefeller Institute for Medical Research, New York.

A bacteriophage active against certain mucoid strains of hemolytic streptococci, originally isolated by Clark and Clark[1], was used in tests with a series of 119 strains of hemolytic streptococci which had been obtained from a variety of sources, some pathological and some normal. Of these, 37 strains were isolated from human sources, including 5 throat cultures from normal persons; 42 strains were recovered from mastitis in cows and from milk and cheese; 18 from lymphadenitis in guinea pigs; 7 from spontaneous rabbit infections with hemolytic streptococcus; 7 from pleuro-pneumonia, chronic endometritis and distemper in horses; 2 from pneumonia in

[1] Clark, P. F., and Clark, A. S., *J. Bact.*, 1926, **11**, 89; PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 635.

foxes; 3 from streptococcus infections in swine; and 3 from a disease known as "slipped tendon" in chickens.

TABLE I.

Susceptibility of Strains of Hemolytic Streptococcus to Streptococcus Bacteriophage (Clark).

Source of strains	Susceptible No.	Susceptible Serological group	Insusceptible No.	Insusceptible Serological group	Total
Human	6*	A	31	A, B	37
Bovine	14	C, E	20	A, B	34
Cheese			8	D	8
Guinea pig	18	C			18
Rabbit	5	C	2	A, B	7
Horse	6	C	1	C	7
Fox	2	C			2
Swine	2	C	1	C	3
Chicken	3	C			3
Total	56		63		119

The tests were performed by adding 1 cc. of a rapidly growing 2-hour broth culture to 1 cc. of bacteriophage. The phage was either the Berkefeld filtrate of a lysed culture or the unfiltered, crystal clear, lysed culture. Controls of culture plus broth were also included. The tubes were incubated for 1 to 18 hours at 37°C.; but lysis was usually complete in one hour if it occurred at all. Overgrowth of resistant forms frequently occurred if the tubes were incubated over night.

* The strains of human origin were only slightly susceptible to bacteriophage.

The table shows the number of strains of hemolytic streptococcus which were susceptible to the Clark bacteriophage and the number insusceptible, as well as the source of the cultures. All susceptible strains, except the 6 of human origin, were strikingly affected by the action of this phage, and were further characterized by the formation on blood agar plates of mucoid colonies with exceptionally large zones of hemolysis. The susceptible human strains, on the contrary, did not form mucoid colonies, and were only slightly sensitive to the action of the phage and with much less regularity than the strains of animal origin.

The addition of bacteriophage to susceptible cultures did not lead to the formation of plaques or "moth-eaten" colonies. This observation is in accord with the findings of others who have worked with bacteriophage active against hemolytic streptococci isolated from guinea pigs, and have rarely or never found such colonies.[1, 2, 3]

Without the addition of bacteriophage, however, one of the strains in the present series occasionally showed "moth-eaten" colonies. Some further suggestion of the association of a lytic

[2] Cunningham, J. S., *J. Inf. Dis.*, 1929, **45**, 474.

[3] Colvin, M. G., *J. Inf. Dis.*, 1932, **51**, 17.

agent with the susceptible cultures, except those of human origin, was also indicated by the formation of transparent colonies as the cultures aged, although the complete disappearance of the colonies from plates was not observed. The presence of a lytic principle in these cultures was also suggested by the fact that, after a few rapid 2-hour subcultures in broth, several strains underwent spontaneous lysis, which was transmissible in series and caused lysis of broth cultures of other strains without the addition of bacteriophage from any other source. These cultures were of guinea pig origin, but no systematic investigation was undertaken to determine the possible association of spontaneous lytic properties with strains isolated from other animals.

The bacteriophage, used in the tests recorded here, was obtained by Clark and Clark from sewage, and was highly active against 2 mucoid strains of hemolytic streptococcus isolated by them from spontaneous lung infections in a rabbit and a guinea pig. Because of an unpublished observation of Dr. A. G. Kuttner, made in 1926, when the Clark phage was brought into this laboratory, that all strains of hemolytic streptococci isolated by her from spontaneous guinea pig infections were lysed by this bacteriophage, the cultures in the present series were tested for susceptibility to the same lytic agent, which was recovered from a Berkefeld filtrate kept sealed during the intervening years.

Of 119 strains of hemolytic streptococci isolated from various sources, 50 were found susceptible to lysis by bacteriophage. Fifty of these strains were especially susceptible to phage and were all derived from lower animals. These observations were made in connection with a study dealing with a serological differentiation of human and other groups of hemolytic streptococci. While no definite relationship was found to exist between the serological specificity and the susceptibility of these organisms to bacteriophage, it is, nevertheless, interesting that, of the 50 strains susceptible to phage, 30 fell into 2 sharply defined serological groups: 27 in group C and 3 in group E. All 50 of these were of animal origin. The letters in the table indicate the serological groups to which the strains belonged, and the basis of that grouping will be the subject of a separate paper.

6410

Presence of Capsules on Bacterium Granulosis.*

J. W. CHURCHMAN AND N. V. EMELIANOFF.

From the Laboratory of Experimental Therapeutics, Cornell Medical College.

Noguchi[1] by the use of special methods isolated from cases of human trachoma a new bacterial species to which the name *Bacterium granulosis* was given. The organism was described as a small, motile, monotrichous, Gram negative rod; no mention was made of a capsule. With it a chronic granular conjunctivitis was induced in *Macacus rhesus;* and the disease thus experimentally produced was transferred by direct tissue passage to the chimpanzee, baboon and other *Macacus rhesus* through at least 4 successive passages. *Bacterium granulosis* was regarded by Noguchi as the probable inciting microorganism of trachoma and its equivalent, granular conjunctivitis in monkeys. The rôle played by the organism in the production of human trachoma has since been experimentally reviewed by a number of other investigators. General agreement on the subject has not been reached but there is a fairly large body of evidence to support Noguchi's contention.

We have recently studied this organism by the new technic for staining capsules recently described,[2] using for this purpose a culture obtained by Olitsky and Tyler[3] from a case of human trachoma. Capsules were found on both the S type, and the R type dissociated by Tyler. The capsules could also be demonstrated by the Casares Gil method for staining flagella. When this stain was used the capsular attachment of the flagella, as previously described by us,[4] was clearly demonstrable.

* Work aided by a grant from the Josiah Macy, Jr., Foundation.

1 Noguchi, H., *J. Exp. Med.*, 1928, **18**, 1.

2 Churchman, J. W., and Emelianoff, N. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 514, 515, 996.

3 Olitsky, P. K., and Tyler, J. R., *Science*, 1930, **71**, 564.

4 Churchman, J. W., and Emelianoff, N. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 996.

6411

Avitaminosis XIII. Effect of Vitamin A Deficiency and Vitamin D Deficiency on Differential Leucocyte Count of the Albino Rat.*

BARNETT SURE AND KATHRYN S. BUCHANAN.

From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.

Vitamin A Deficiency. Forty-nine rats were employed, 30 to 50 days of age, each animal weighing 50 to 86 gm. Of these, 31 were placed on a diet satisfactory in every respect with the exception of vitamin A, and 18 served as controls. The experimental period lasted 30 to 70 days. Various stages of the avitaminosis were produced, ranging from loss of weight with or without accompanying incipient ophthalmia to severe ophthalmia associated with pneumonia. Our results on the differential leucocyte count in vitamin A deficiency are essentially in accord with the recent findings of Turner and Loew,[1] namely, that, as the disease progresses in severity there is a relative increase of polymorphonuclear leucocytes and a corresponding decrease in lymphocytes in the majority of pathological animals compared with the litter mate controls. The extent of such relative polymorphonuclear leucocytosis and corresponding lymphopenia is 10 to 30%. What we are unable to explain, however, is why a number of animals that are apparently in advanced stages of this avitaminosis show no significant changes in the differential leucocyte count.

Total leucocyte counts were made on 6 pathological animals and on 6 controls. The extent of variation on the same control animals was so great that no noteworthy influences can be ascribed to the vitamin A deficiency. In this respect our findings are not in agreement with those of Turner and Loew who report higher values for animals suffering from this avitaminosis. No significant differences were observed in the monocyte, basophil or eosinophil counts between the pathological and control animals.

Vitamin D Deficiency. For the vitamin D work 42 animals were employed, 31 of which were placed on Steenbock and Black's ricketic diet,[2] and 11 of which served as controls. The animals

* Research paper No. 270, Journal Series, University of Arkansas.

1 Turner, R. G., and Loew, E. R., PROC. SOC. EXP. BIOL. AND MED., 1931, **28**, 506.

2 Steenbock, H., and Black, A., *J. Biol. Chem.*, 1925, **64**, 263.

were 29 to 48 days of age when placed on the experiment and each weighed 40 to 50 gm. The experimental period ranged from 30 to 35 days.

A summary of all the results discloses that in experimental rickets produced on diets high in calcium and low in phosphorus, and deficient in vitamin D, no disturbance takes place in the differential leucocyte count. No observations were made on the total leucocyte count in this avitaminosis.

6412

Avitaminosis XII. Influence of Vitamin B Deficiency on Differential Leucocyte Count of the Albino Rat During Lactation.*

BARNETT SURE AND KATHRYN S. BUCHANAN.

From the Department of Agricultural Chemistry, University of Arkansas, Fayetteville.

It was reported that in vitamin B deficiency of the weaned albino rat, there is produced a relative lymphopenia and corresponding polymorphonuclear leucocytosis[1] which is due to the *specific* effect of this avitaminosis[2] unrelated to the plane of nutrition.

In this investigation a study was made of the influence of vitamin B deficiency on the differential leucocyte count of the lactating albino rat and its nursing young. Forty-seven mothers and litters were employed, 21 of which were pathological and 26 of which served as controls. The biological methods used have been previously described.[3] The experiments were set out in pairs and triads, so that the results could be interpreted as due to inanition or to the vitamin B deficiency *per se*.[3]

In lactation studies of the rat complicating factors arise that are not encountered in growth. For instance, since it is impossible to obtain sufficient milk from nursing rats for biological studies, the criterion of lactation performances is the character of growth of the nursing young, which is correlated with a limiting factor in

* Research paper No. 269, Journal Series, University of Arkansas.

1 Sure, B., Thatcher, H. S., and Walker, D. J., *Arch. Path.*, 1931, **11**, 413.

2 Sure, B., and Walker, D. J., *Arch. Int. Med.*, 1932, **49**, 405.

3 Sure, B., *J. Biol. Chem.*, 1932, **97**, 133.

the diet. Satisfactory technique has not yet, however, been perfected, so that it is possible to know, in the later stages of the nursing period, to what extent the nurslings are deriving their nourishment from the mother's milk and to what extent their daily nutritional requirements are being supplemented by the maternal diet which is accessible to them. For such reasons, we frequently encounter irregular results in biochemical studies of avitaminotic rats during lactation. A summary of all the results discloses that in vitamin B deficiency there is generally a relative lymphopenia and corresponding polymorphonuclear leucocytosis in the lactating albino rat and nursing young, and, while in some groups a *specific* effect of vitamin B deficiency on such changes was apparent, in the majority of cases this was due to great losses of weight associated with inanition. No noteworthy changes were observed in the monocyte, eosinophil or basophil counts.

6413

Comparison of Bone Ash of Rachitic Rats Treated with Viosterol and with Phosphate Ion.

C. A. LILLY. (Introduced by L. H. Newburgh.)

From the Department of Internal Medicine, Medical School, University of Michigan.

A group of 21 albino rats, 28 to 30 days old, were placed in individual cages and fed Steenbock's rachitogenic Diet No. 2965, *ad lib.* Twenty-eight days later they were found to have X-ray evidence of florid rickets. They were then divided into 3 groups of 7 rats each.

GROUP I. Diet not changed. This was the control group.

GROUP II. Diet modified as follows: 0.3 gm. Viosterol (potency 250 D) was thoroughly mixed through 50 cc. of boiled, but partially cooled Crisco, which was in turn thoroughly rubbed into each 950 gm. of the Steenbock Diet and fed *ad lib.* This amount of Viosterol provided the animal with an equivalent of 3% of cod liver oil in the diet.

GROUP III. Diet not changed, but each day each animal was given 1 cc. of a solution of 43.4 gm. Na_2HPO_4, 12 H_2O, in 500 cc.

distilled water.* One cc. of this solution contains the phosphorus equivalent contained in 10 gm. of the Steenbock Diet No. 2965 plus 0.4% K_2HPO_4. We assumed that each rat ate 10 gm. of diet daily. Hess[1] states that the addition of 0.4% secondary phosphate prevents rickets when added to a rachitogenic diet.

The animals were all confined in a dark room, the drinking water being distilled.

The animals all lived, but one rat in Group I and one in Group II lost weight and were discontinued as test rats.

The respective diets of each group were continued for 28 days, when all the animals were killed, the femurs removed, cleaned, weighed and ashed.

RESULTS:

Group I. Bone ash, % of wet weight = 28
Group II. Bone ash, % of wet weight = 45
Group III. Bone ash, % of wet weight = 45

The importance of an adequate mineral supply in the reconstruction of rachitic bones has not been sufficiently stressed in the past. It is demonstrated that the addition of Na_2HPO_4 in adequate amounts to a low phosphorus rachitogenic diet produces a bone ash comparable to the bone ash produced by adding pure vitamin D to such a diet.

6414

Failure to Produce Dental Caries with High Carbohydrate, and with Extremely Low Fat Diets.

C. A. LILLY AND J. D. GRACE. (Introduced by L. H. Newburgh.)

From the Department of Internal Medicine, Medical School, University of Michigan.

A. It is frequently stated that a high carbohydrate diet tends

* The Na_2HPO_4 was given as follows: Each rat was removed from the cage, the thumb and index finger of the left hand firmly, yet gently, grasping the skin of the nape of the neck, while the ring and middle finger secured the fore legs. The rat was thus securely held with its back in the palm of the left hand. The solution of Na_2HPO_4 was then introduced drop by drop well back in the rat's mouth by means of a one cc. curved medicine dropper held in the right hand. This procedure requires much kindness and patience, and often as long as 15 minutes are consumed in getting 1 cc. of fluid "down" the rat.

[1] Hess and Unger, *J. Biol. Chem.*, 1922, 50.

to produce dental caries. To test the truth of this statement, the following experiment was performed. Thirty albino rats, 30 days old, were removed from the breeding cages and placed in individual cages. They were then divided into 3 groups.

Group I was fed, *ad lib.*, a diet of glucose 66%, casein 20%, lard 10%, Mendel's salt mixture 4%. Into this diet 0.3 gm. Viosterol (potency 250 D) was thoroughly mixed through the melted, but partially cooled lard of the diet, and thoroughly rubbed through each 1000 gm. of the diet. In addition, each day, each rat was given 0.4 gm. of dried brewer's yeast and a piece of fresh lettuce. Thus adequate vitamins were furnished.

Group II received the same diet, except that lactose was substituted for the glucose.

Group III received the same diet, except that maltose was substituted for glucose.

The animals all grew normally. However, some of the animals in Group I (glucose) had diarrhea at intervals during the experiment. The animals all lived except one rat in Group I, and one rat in Group III, which died of an undetermined cause.

After conducting the test for 6½ months, the rats were killed, the heads cleaned, and the teeth examined under light and magnification for caries. No caries was found.

B. It is sometimes also stated that an extremely low fat diet tends to produce caries. As little has been reported relative to the influence of an extremely low fat diet on dental caries, the following experiment was performed: Ten albino rats, 4 to 5 weeks old, were placed in individual cages, in a well lighted large room. The animals were divided into 2 groups of 5 rats each.

Group I was given, *ad lib.*, a diet of casein 19%, starch 73%, Mendel's salt mixture 3%, cod liver oil 5%. Fresh lettuce was also given 6 times a week for its vitamin content.

Group II was given, *ad lib.*, the same diet, but butter fat* substituted for cod liver oil.

The animals all grew to or above normal weights. All lived with the exception of one in Group II, which died after being on the test for 5½ months.

The feeding was continued 9 months, when the animals were killed, the heads cleaned, and the teeth examined for caries. No caries was found.

Conclusions. High carbohydrate diets of glucose, lactose, and maltose, and extremely low fat diets (butter fat and cod liver oil) failed to produce dental caries in albino rats.

* The butter fat was a centrifuged preparation.

6415

Temperature Reaction Following Intravenous Injections of Proteins of Navy Bean with Special Reference to Hemagglutinating Fraction.*

ROSEMARY ANNE MURPHY, ADA R. HALL AND VERZ R. GODDARD.

From the Department of Zoology and Physiology, Wellesley College.

Hemagglutinin preparations from seeds of *papilonaceae, i. e.,* beans, peas, lentils and vetch, were first made by Landsteiner and Raubitschek.[1] Because these substances did not produce death when relatively large amounts were injected intraperitoneally into mice and guinea pigs, they were designated as "non-toxic". Since the hemagglutinating fraction from navy beans has been used as an agent for ridding anti-sera of suspended erythrocytes[2] and as it is capable of being highly purified without being rendered insoluble,[3] it is of interest to learn whether it causes the production of a fever upon injection. The results here reported give the effect upon the rectal temperature curve of rabbits of intravenous injections of the hemagglutinin prepared from the navy bean.

Fifteen rabbits were used for the injections which were made in 5 cc. volumes into the marginal ear vein. The materials tested for pyrogenicity were dissolved in sterile redistilled water prepared by the method outlined by Seibert.[4] The temperatures of the animals, taken by a Faichney rectal thermometer, were recorded at hourly intervals for 7 or more hours. As was found by Seibert and Mendel,[5] the range of temperature variations for individual rabbits was fairly constant for the normal uninjected animals. (Plate I, a. b. c. and d.) The negative reactions of the animals injected with the water used as a solvent are shown by the curves of Plate II, a, b, c, and d. The temperature reactions resulting from the injection of a purified preparation of the hemagglutinin from the navy bean were found to be even stronger than those resulting from injections of egg albumin and peptone solutions. (Plate III.) The preparation

* The data reported in this paper are taken from the thesis presented by Rosemary Anne Murphy in partial fulfillment of the requirements for the degree of Master of Arts, Wellesley College, 1931.

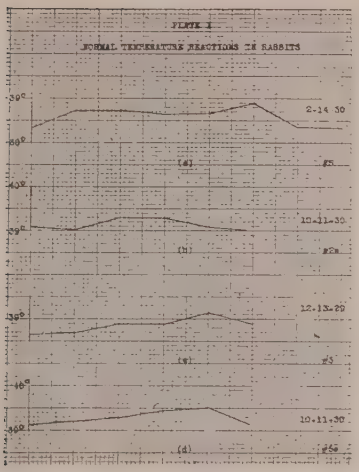
1 Landsteiner and Raubitschek, *Centbl. Bakt.*, 1907, **45**, 660.

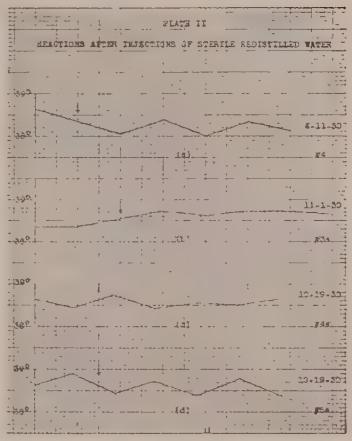
2 Dorset and Henley, *J. Agric. Research*, 1916, **6**, 333.

3 Goddard and Mendel, *J. Biol. Chem.*, 1929, **82**, 447.

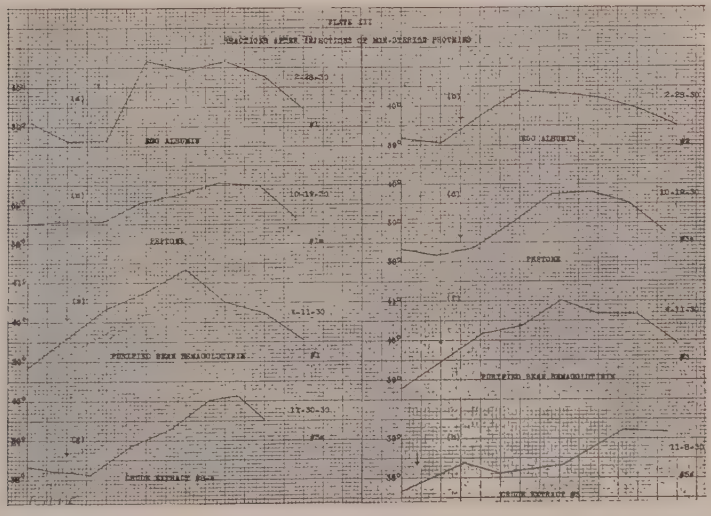
4 Seibert, *Am. J. Physiol.*, 1923, **67**, 90.

5 Seibert and Mendel, *Am. J. Physiol.*, 1923, **67**, 83.





of hemagglutinin used had been stored for 5 years in powder form and carefully protected from moisture. No attempt had been made either to prepare or to keep it in a sterile condition. Freshly prepared crude extracts of the hemagglutinin of the navy



bean were found to be on the whole less pyrogenic than the highly purified material which had been stored. Sterile redistilled water was used in making these extracts. After having been washed in the non-pyrogenic water, the beans were ground in a sterile mortar and

TABLE I.

Temperature Reactions Following Intravenous Injections of Proteins.

Date	Protein Injected	Amt. Injected mgm.	No. of Animals	Temperature Variation °C. +	Temperature Variation °C. —	Solvent* Water
2- 7-30	Egg Albumin	5	4		0.55	B
	" "	5	5	1.11		B
2-28-30	" "	5	1	2.01		C
	" "	5	2	1.33		C
3- 7-30	Purified Hemagglutinin	5	4	2.45		B
	" "	5	5	2.22		B
4-11-30	" "	5	1	2.50		D
	" "	5	3	2.23		D
10-19-30	Peptone	500	1a	1.05		AA
	"	500	3a	1.60		AA
11- 8-30	Crude Extract No. 5	—	2a	0.84		BB
	" " "	—	4a	0.77		BB
	" " "	—	5a	1.56		BB
	" " "	—	6a	0.78		BB
11-30-30	" " No. 8-a	—	5a	2.00		DD1
	" " "	—	6a	0.94		DD1

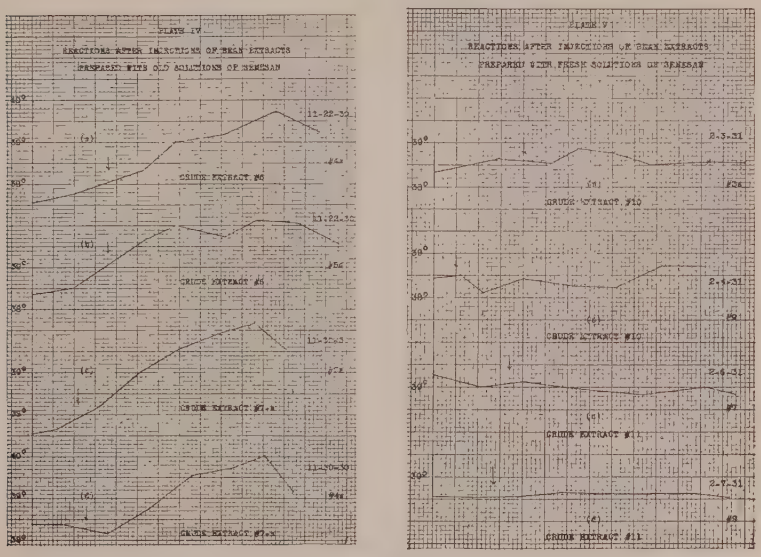
* These are non-pyrogenic, distilled waters, prepared according to the method of Seibert and Mendel.[9]

soaked in 4 times their weight of water for 15 hours at 5°C. The supernatant fluid, which was poured off and injected, contained some starch grains as well as proteins and traces of other substances extractable from the bean. Table I gives the temperature variations following these injections, compared with those resulting from injections of other protein solutions mentioned above.

Working on the assumption that a protein at its source would be free from bacterial contamination, an attempt was made to obtain a non-pyrogenic protein solution by extraction of the beans after the outer coating had been sterilized. A commercial seed sterilizer known as Du Bay Semesan was used. Its composition is given on the package as 30% hydroxymercurichlorophenol and 70% inert ingredients. A 0.25% solution of this material was used. The beans were soaked in Semesan for one hour to sterilize the seed coat. The poison could then be completely removed by washing the beans in the sterile redistilled water. The hydroxy-phenyl group of the sterilizer was readily detected by Millon's reagent for this grouping. Washings at hourly intervals were continued for some 5 hours after a negative Millon's test resulted.

From beans sterilized in accordance with the method indicated and extracted with non-pyrogenic water, extracts were obtained which did not cause fever upon injection. Good results were obtained, however, only when a *freshly prepared* solution of sterilizer

was used. Semesan solutions a week or more old were ineffective in preventing strongly positive fever reactions when the extracts were injected. Plates IV and V draw a comparison between the



PLATES I-V.

The date of experiment and the number of animal are found with each curve. Arrows indicate time at which injections were made. One cm. abscissa equals 30 min. One cm. ordinate equals 0.5°C.

temperature reactions following the use of old Semesan solutions, with those from beans sterilized with freshly prepared solutions.

To eliminate the possible criticism that the non-pyrogenicity of the extracts might be ascribed to the fact that the protein present was insufficient in quantity to produce a fever, analyses were made of the amounts of protein present in 5 of the crude extracts. Nitrogen was determined by a modified *Folin-Farmer Micro-Kjeldahl* method.[6] The nitrogen figure was multiplied by the factor 6.25 for the protein estimation. The amounts found (13-17 mg.) varied considerably as a result, probably, of differences in the extent to which the beans were ground in the process of preparing the samples. In every case, however, the amount injected far exceeded the weights of proteins injected in the fever-producing solutions listed in Table I with the exception of the peptone solution. They also exceeded

[6] Hall, unpublished data.†

† Modification of the Folin-Farmer Micro-Kjeldahl method.

the amounts which Seibert and Mendel injected[7] to produce characteristic fevers.

The results give additional support to the conclusion of Seibert[4] that bacteria are responsible directly or indirectly for the production of fever upon the introduction into the circulation of substances foreign to it. Her conclusions came as the result of an exhaustive study of the fever-producing substances in distilled waters. The work of Rademaker[8] likewise confirms her hypothesis. Barkan and Nelson[9] studied the cause of the febrile reactions following injections of milk and concluded that bacteria or products of bacterial action and not the proteins of the milk were pyrogenic. The work reported here indicates that the proteins of the navy bean are not pyrogenic *per se* but that they are easily contaminated. Injected extracts prepared from navy beans produce a slight or a strong fever reaction, depending upon the degree of bacterial contamination.

Summary. A method is presented for preparing a strongly hemagglutinating, non-pyrogenic extract from navy beans. This extract is high in protein content. The method demonstrates that the proteins of the bean which may be extracted by water are not fever-producing unless contaminated by bacteria or products of bacterial action.

6416

Oxygen Consumption by Acidified Tissues.

JOSEPH NEEDHAM. (Introduced by William R. Amberson.)

From the Biochemical Laboratory, Cambridge, England.

Amberson, Armstrong and Root[1] have described a curious phenomenon; the persistence of oxygen consumption without carbon dioxide production in acidified tissues. *Fundulus* eggs, for instance, continued to take up some 7 to 14% of the oxygen which they had previously been consuming in normal respiration. This residual oxygen-uptake persisted after neutralization of the acid, and was not affected by KCN or by high temperatures.

In recent experiments[2] on the respiration and respiratory quotient

[7] Seibert and Mendel, *Am. J. Phiysiol.*, 1923, **67**, 105.

[8] Rademaker, *Ann. Surg.*, 1930, **92**, 195.

[9] Barkan and Nelson, *J. Am. Med. Assn.*, 1924, **82**, 190.

[1] Amberson, Armstrong and Root, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 31.

[2] Needham, *Proc. Roy. Soc. London*, B, 1932.

of the embryo and the extra-embryonic membranes of the hen's egg, a similar phenomenon has been observed. These experiments were carried out in Warburg manometers fitted with the cups designed by Dickens and Simer,[3] and the procedure in determining a respiratory quotient was essentially that laid down by the latter investigators. Three manometers are used, 2 of which contain similar pieces of tissue, while the third contains the solutions alone. The cups have an annular trough containing baryta, and a side-bulb containing 2.5N HCl. Manometer 1 gives the oxygen-uptake of its tissue by a steadily increasing negative pressure, and when the acid is tipped at the end of the experiment, the carbon dioxide due to respiration, that bound in the tissue at the beginning, and that contained in the solutions, give together a large positive pressure. The 2 non-respiratory quotas of carbon dioxide are given by manometers 2 and 3 respectively, which are tipped at the beginning of the experiment and show, in one case, the carbon dioxide of tissue and solutions together, in the other case, the carbon dioxide of solutions alone.

The phenomena here reported were often seen in manometer 2, for although respiration was expected to cease on acidification, it was common, though by no means invariable, to note a continuous small development of negative pressure. Thus, to take a typical instance (Exp. 357, 5-day chick embryo in Ringer-phosphate-glucose medium, atmosphere of pure oxygen, bath at 36°), the bound CO_2 in tissue and solutions gave at the beginning of the experiment, a positive pressure of +2.15 cm. Brodie fluid. But during the succeeding period of 4 hr. 35 minutes, during which the manometer 1 was developing a negative pressure of —6.95 cm. the acidified cup also developed a negative pressure amounting to —0.55 cm. As the weights of the embryos were almost identical, the "respiration" of the acidified embryo was about 7% of the normal one, *i. e.*, a residual oxygen consumption of exactly the same order as that reported by Amberson, Armstrong and Root.

The effect has been observed not only with avian tissues (embryo up to the 6th day of incubation, yolk-sac, blastoderm), but also with the eggs and embryos of the shore-crab, *Carcinus moenas*. Amberson, Armstrong and Root do not offer any explanation for it, but the following possibilities should probably be kept in mind. Oxygen-consumption without carbon dioxide production may theoretically arise from (a) the oxidation of organic sulphur to sulphate, (b) the transformation of lactic to pyruvic acid, and of glucose to

[3] Dickens and Simer, *Biochem. J.*, 1930, **24**, 905.

glycuronic acid, (c) the formation of acetoacetic acid from fat, (d) the oxidation of reduced glutathione, (e) the oxidation of lipoids (*c. f.* their spontaneous decomposition in air). No doubt the effect is due to a combination of these factors.

6417

Resistance of Glucose Urea to Urease and Other Enzyme Action; Non-Absorbability of Glucose Urea from the Jejunum.

F. A. CAJORI. (Introduced by D. Wright Wilson.)

From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania.

In studying the mechanism of urease action, it would be of interest to find a derivative of urea that would be hydrolyzed in the presence of urease. Urease is very specific in its action and urea appears to be its only substrate. Closely related compounds such as amides or purines as well as simple derivatives of urea are not attacked by this enzyme. Armstrong and Horton[1] found that substitution in the urea molecule with methyl or ethyl groups invariably rendered the substituted urea inaccessible to urease. Schoorl[2] synthesized glucose-urea, in which one molecule of urea was united with one molecule of the sugar. This compound is very soluble in water and is stable in solution. When heated with acid it undergoes hydrolysis with the formation of δ-glucose and urea. Johnson and Bergmann,[3] in their recent researches on nitrogenous glucosides have prepared glucose urea, and Dr. Johnson generously placed at our disposal a very pure sample of glucose urea for investigation. We have studied the action of urease on this urea derivative.

Jack bean urease was found to have no ability to decompose glucose urea. There was no ammonia production in 10 minutes when urease was added to 0.2 M solutions of this compound. However, if glucose-urea was hydrolyzed by acid prior to the addition of urease, ammonia production occurred at a rate comparable to that observed when a 0.2 M solution of urea and glucose was exposed to urease. The results (Table I) clearly demonstrate that the inability of urease to split glucose urea must be ascribed to the chemical

[1] Armstrong, E. F., and Horton, E., *Proc. Roy. Soc.*, Series B, 1912, **85**, 109.
[2] Schoorl, M. N., *Rec. Trav. chim. Pays-Bas*, 1903, **22**, 1.
[3] Johnson, T. B., and Bergmann, W., *J. Am. Chem. Soc.*, 1932, **54**, 3360.

TABLE I.
Urease Action on Glucose-Urea. 5 cc. Substrate (0.2 M), 5 cc. KH_2PO_4 (0.1 M), 0.5 cc. Urease Solution.

Exp.	Substrate	T°	Time	NH_3 Formed
			min.	cc. 0.1 N
I	Glucose urea	22°	10	0.01
	" "	"	10	0.04
	Urea + glucose	"	10	10.68
	" "	"	10	10.55
II	Glucose urea	25°	7	—0.02
	Urea + glucose	"	7	3.26
	Hydrolyzed glucose urea	"	7	4.35
	" " "	"	7	4.36
	Control. Hydrolyzed glucose urea. No urease	"		0.31
III	Hydrolyzed glucose urea	27°	4	4.83
	" " "	"	6	6.93
	Urea + glucose	"	4	5.83
	" "	"	6	8.54
IV	Urea + glucose urea (0.2 M)	23°	8	8.01
	" " " (0.1 M)	"	8	8.24
	" "	"	8	8.21
	" "	"	8	7.78

make-up of the compound and cannot be the result of the presence, in the preparation, of substances that inactivate the enzyme. With the sugar group attached, urea cannot form a configuration which urease can attack. A substituting group, be it methyl, ethyl or glucose protects the urea from the enzyme. In the presence of glucose urea, urea decomposition by urease was not inhibited.

Glucose urea was also found to be unfermented by bakers' yeast. Neither emulsin (β glucosidase) nor yeast maltase (α glucosidase) caused any hydrolysis of glucose urea and no cleavage of this compound occurred in the presence of nucleosidase, prepared from pig kidneys by the method of v. Euler and Brunius.[4] In agreement with the results of Mayer,[5] we found that when this substance was injected into the blood stream of a rabbit it was largely (70%) excreted, unchanged, in the urine.

Glucose urea, despite its solubility and relatively low molecular weight, is not absorbed from an intestinal loop of a dog. The absorption of glucose urea was studied by inserting a solution of this compound in a Thiry loop of a dog's jejunum and after a suitable interval removing the contents of the loop. Through the courtesy of Drs. Ravdin and Johnston of this Medical School, an animal surgically prepared by them was used and their technique followed for carrying out absorption studies. After washing the loop with water, 20 cc. of a 0.09 M solution of glucose urea was inserted in

[4] v. Euler, H., and Brunius, E., *Ber. Chem. Ges.*, 1927, **60**, 1584.
[5] Mayer, P., *Biochem. Z.*, 1909, **17**, 145.

TABLE II.

Recovery of Glucose Urea from a Jejunal Loop of a Dog. 20 cc. of .09 M glucose urea, equivalent to 324.2 mg. of glucose remained in the loop 35 minutes.

Vol. fluid removed	Reducing Substances as glucose*		Glucose urea removed	Glucose urea hydrolyzed in the loop
	Before Hydrolysis	After Hydrolysis		
cc.	mg.	mg.	%	%
23	0	315	97	0

*Corrected for reducing substances removed from the loop during a control experiment.

the loop. At the end of 35 minutes, the contents of the loop were removed and the loop thoroughly washed, and analyzed for reducing sugar, before and after hydrolysis, by the Hanes method.[6] Correction was made for the small quantity of reducing substances that accumulated in the loop when 20 cc. of physiological saline was introduced into the loop for 35 minutes. The results of this experiment (Table II) show an almost quantitative recovery of glucose urea and are in marked contrast to results observed when a solution of glucose[7] or sucrose[8] was inserted into this loop. These sugars were rapidly and in large part absorbed in the course of a half hour. It may be concluded that glucose urea was not absorbed from the dog's jejunum. Further, no glucose urea was hydrolyzed during its exposure to intestinal enzymes.

The presence in blood or other tissues of any appreciable quantity of urea united with glucose, as glucose urea, seems unlikely since all the urea present in blood is decomposed by urease and the presence in blood filtrates of a non-fermentable substance that yields glucose on hydrolysis has not been demonstrated.

6418

Glycogen Content of the Rat Heart.

C. N. H. LONG AND* G. T. EVANS. (Introduced by J. C. Meakins.)

From the University Clinic, Royal Victoria Hospital and McGill University, Montreal.

The cardiac glycogen of albino rats has been determined under a variety of conditions. The figures quoted are for glycogen as glu-

[6] Hanes, C. S., *Biochem. J.*, 1929, **23**, 99.

[7] Unpublished results of Ravdin and Johnston.

[8] Unpublished results of the author.

* With the aid of a grant from the Banting Research Foundation.

cose in milligrams percent, and unless otherwise stated are for 24-hour fasted animals. Amytal anesthesia was used when securing hearts and muscles.

Fifty-two 24-hour fasted animals, used as controls, showed a quite constant cardiac glycogen of 497±56 mg. %; the gastrocnemii of the same animals contained 525±48 mg. %.

Animals fasted for 48 hours had more glycogen in the hearts (578±81) and less in the gastrocnemii (455±37) than controls.

Non-fasted animals showed considerably less cardiac glycogen (341±54) but more glycogen in gastrocnemii (574±74) than controls.

It was found also, that 24-hour fasted animals which had been fed sufficient glucose by mouth to provide for maximum absorption for 4 hours, and which were taken at the end of such time, had somewhat less glycogen in the hearts (449±50), although considerably more in the gastrocnemii (690±48) than controls. When, however, insulin injection accompanied such glucose feeding, both hearts and gastrocnemii contained increased glycogen (hearts 703±212, gastrocnemii 830±183).

Epinephrine sufficient to reduce the glycogen of gastrocnemii to 55% of its control value did not alter cardiac glycogen appreciably. The cardiac glycogen after subcutaneous injection of 0.02 mg. of epinephrine per 100 gm. of rat, was (a) in ½ hour 475±52, (b) in 3 hours 509±54. One-half hour after large intravenous doses of epinephrine the hearts contained 475±26.

Similarly, exercise, both natural and electrically produced, sufficient to reduce the glycogen of gastrocnemii to 70% of its control value did not decrease cardiac glycogen, the values being (a) after natural exercise 546±40, (b) after electrical stimulation 512±19. When, however, electrical stimulation was severe enough to produce cyanosis through interference with respiratory movements the cardiac glycogen was much reduced, being 226±33. This finding is in agreement with those recorded below under anoxaemia.

Marked changes in the $H_2CO_3/NaHCO_3$ ratio of the blood can occur without altering greatly the cardiac glycogen. Thus, after 3 hours in an atmosphere of 8% CO_2, 92% O_2 animals had a glycogen content in hearts of 458±27, and in gastrocnemii of 532±32. Four hours after sufficient $NaHCO_3$ by mouth to produce a plasma CO_2-combining power of from 76 to 88 vol. % the animals had a cardiac glycogen content of 466±31 and in gastrocnemii of 517±46. When, however, larger doses of $NaHCO_3$ were given, producing a CO_2-

combining power of 108 to 123 vol. % and resulting in tetany, the glycogen content of both hearts and gastrocnemii was much reduced (hearts 261, gastrocnemii 178).

Anoxaemia readily lowers cardiac glycogen. Immediately after asphyxia by coal gas, hearts were found to contain only 25 mg. %. The hearts of animals 2 minutes after clamping the trachea contained 83 mg. %. Less acute experiments were done by placing the animals for 3 hours in atmospheres containing low percentages of oxygen. At the end of such time in 12% to 7.4% O_2 no significant change in cardiac glycogen was found: the glycogen of gastrocnemii was not altered in this nor in the lower oxygen percentages which follow. After 3 hours in 6.2% O_2 the cardiac glycogen was 339±101, and in 5.8% it was 221±52. When the oxygen was reduced further, some of the animals did not survive, although 3-hour survivals occurred in as low as 4% O_2. Between 5.8% and 4% O_2, the cardiac glycogen of survivors was 192±65. Those which did not survive, died of cardiac failure with surviving respiratory movements: the hearts of dying animals caught during the period when respirations still persisted, and including 3 with ventricular fibrillation, had glycogen values averaging 85±15. From these experiments alone it can not be concluded that low cardiac glycogen stands in causal relation to cardiac failure, but it is established that there is a sharp difference in the level of cardiac glycogen in hearts which survive and those which do not survive severe grades of anoxaemia.

The recovery process following anoxaemia is rapid and complete. Thus immediately after 3 hours in 5.8% O_2, cardiac glycogen was found to be 221±52, whereas after ¾ hour recovery from such it was 400±24, after 1 hour recovery 485±17, after 1½ hour recovery 484±38 and after 3 hours 547±20.

Single gastrocnemii were exercised by a uniform electrical stimulation. Immediately after such exercise, the glycogen content was 214±49. Others allowed to recover contained (a) in ½ hour 357±51, (b) in 1 hour, 407±49, (c) in 2 hours 430±47. It is to be observed that the recovery in a single exercised gastrocnemius, which has approximately the same weight as a heart, is not so rapid or complete.

A discussion of the significance of these findings is considered out of place at present. Two observations, however, are thought important: (1) Consistent values for cardiac glycogen in the albino rat have been obtained both for 24-hour fasted controls and for a variety of conditions. (2) In view of (a) the well maintained level of cardiac glycogen under such conditions as fasting, epine-

phrine injection, alkalosis, acidosis, and exercise and (b) the prompt recovery of glycogen after it has been lowered by anoxaemia, it would seem that there exists a quite efficient mechanism for the maintenance of cardiac glycogen.

6419

Unilateral Overflexion of the Limbs Following Experimental Lesions in the Pons and Upper Medulla.*

A. D. KELLER AND W. K. HARE.

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

A characteristic and constant unilateral overflexion of the limbs, during progression or isolated movement of the limbs, has been observed in 12 to 15 cats following hemisection of the brain-stem at the level of the pons or upper medulla—the vestibular nuclei being uninvolved. The abnormal action occurs in the limbs on the opposite side from the lesion. Maximal rather than graded flexion occurs at the ankle, knee, shoulder, or hip joints. In some instances in progression the flexion was maintained to the extent that the animal fell to the affected side because of the failure of the fore limb to extend in time to catch the weight of the body. This overflexion has been seen to involve a fore limb or a hind limb individually. In cases where the lesion extended across the mid-line so as to involve a small medial segment of the opposite half of the stem the ability of the animal to right and to maintain the righted position was impaired or eliminated. In these cases the overflexion occurred when rhythmic running movements were executed. If the animal was passively held in the righted position, typical walking movements embodying overflexion occurred.

Overflexion did not occur following: (1) hemisection of the stem through the thalamus or mid-brain, or even after complete unilateral removal of the mid-brain. (2) Unilateral or bilateral section of the lateral quarter segments of the pons or upper medulla. It was therefore not due to the absence of a rubro-spinal tract. (3) Hemisection of the spinal cord at upper cervical levels. (4) Injury or removal of the lateral lobes or vermis of the cerebellum.

* This investigation is being continued and extended to dogs and monkeys with the aid of a grant from the Ella Sacks Plotz Foundation.

The observations suggest that this disturbance in muscular movement results from injury to structures located in the medial quarter segments of the pons and upper medulla.

6420

Localization of the Mechanisms for Righting in the Brain-Stem of the Cat.*

A. D. KELLER AND W. K. HARE.

From the Department of Physiology and Pharmacology, University of Alabama School of Medicine.

Magnus and Radamaker[1] have demonstrated the dependency of the body righting reflexes from the body and of the labyrinthine righting reflexes upon the mid-brain. Their experiments indicated the dependency of these reflexes on the red nuclei. Ingram and Ranson[2] recently report intact righting reflexes and movements of progression after bilateral destruction of the red nuclei; our experiments, performed independently and using quite a different approach, verify and extend Ingram's and Ranson's observations.

An unoperated animal exhibits the following reactions: (1) it rights the body in falling through the air a short distance, and lights on its feet; (2) when placed on the side, the fore quarters right after the raising of the head, by a rolling, lifting motion in which the fore limbs do not participate to a great degree. The hind quarters follow the fore quarters in like manner. The head, the fore quarters, or the hind quarters will right independently if the other parts are passively fixed. As soon as righting is completed, progression proceeds. (3) If in the standing position, the hind quarters are grasped and passively rotated to one side, the head and fore quarters resist this action by a compensatory rotation in the opposite direction. (4) If the animal is suspended vertically in the air by the hips, the body hangs straight and the head is dorsi-flexed. (When struggling movements are absent.) (5) Equal resistance

* This investigation is being continued and extended to dogs and monkeys with the aid of a grant from the Ella Sacks Plotz Foundation.

[1] Magnus, "Körperstellung", Berlin, J. Springer, 1924.

[2] Ingram, W. R., and Ransom, S. W., PROC. SOC. EXP. BIOL. AND MED., 1932, 29, 1089.

is met in bowing the body—the vertebral axis—to either the right or the left side.

The above described reactions have been found to be essentially unimpaired after the following operative procedures: (Each procedure has been carried out in 2 or more animals, and in most instances in as many as 6 to 8 animals.†) (1) Transection—or hemisection—of the brain-stem at the cephalic level of the mid-brain. Progression was at first modified in that the body was hung low, resulting from the projection of the shoulder blades above the vertebral column. Normal posture was gradually resumed. (2) Complete section of the fibers crossing the mid-line from the cephalic level of the red nuclei to the middle level of the pons by a narrow medial longitudinal lesion. (3) Unilateral section of the lateral quarter segment of the brain-stem at any level of the pons and upper medulla—thus section of rubro-spinal tract on that side. (4) Bilateral section of the lateral quarter segments of the brain-stem at any level of the mid-brain, pons, or upper medulla—bilateral section of rubro-spinal tracts in the pons and medulla.‡ In the latter cases progression was modified from the normal in that there was a medial crossing of the fore limbs in such a way that the one rubbed slightly across the medial aspect of the other. All placing reactions were absent and there was at first a dragging of the dorsum of the feet in their forward movement, indicating destruction of ascending afferents.

Unilateral removal of the mid-brain, which removes one red nucleus completely and cuts all crossing fibers from the other, or complete hemisection of the pons or upper medulla, altered righting and the distribution of tone as follows: (1) *Direct* righting from the same side of the lesion occurred in the normal manner except the reflex was hyperactive. When the reflex was very hyperactive, the body was over-righted so that the body was turned to the opposite side and rolling occasionally resulted. (2) Direct righting did not occur from the side opposite the lesion. The animal either remained lying on this side or righted *indirectly* by (a) pressing the nose against the floor, (b) extending the uppermost fore leg forward in a grasping attitude, and (c) pulling the undermost fore leg underneath the body. (3) From the righted position progression proceeded or the animal fell to the side of indirect righting. (4)

† The extent of the lesions has been checked carefully by serial section of the block of tissue containing the lesion.

‡ Serial section of the mid-brain demonstrated complete disappearance of the large cells of the red nuclei.

Vertical suspension in the air by the hips resulted in rotation of the head and fore quarters to the side opposite the lesion. (5) Unequal distribution of muscular tone on the 2 sides of the body was evidenced by (a) the tendency of the body to bow to the side opposite the lesion, and (b) a marked resistance to the passive bowing of the body to the side of the lesion.

6421

Injection of Peripheral Nerves from the Subarachnoid Space.

LOUIS MANTELL. (Introduced by W. E. Sullivan.)

From the University of Wisconsin.

The injection mass used by Funaoka and Yamada[1] suggested such interesting possibilities that their work was repeated and extended. For the most part their mixture of ultramarine blue 2 parts, ether 15 parts, and turpentine 30 parts was satisfactory.

The principle of inlet and outlet cannulation was adopted in all of the work. In one group 2 laminectomies were done, one in the cervical region, the other in the sacral. The dura mater was opened and relatively large glass cannulae were introduced into the subarachnoid space. The cervical cannula served as the inlet, the sacral as the outlet. For another group the inlet cannula was placed in the lumbar segment, the severed optic nerves served as outlet cannulae. The pressure was usually from 3 to 5 pounds. Fifteen dogs were used. The injections were usually begun with the animal under an anesthetic. They died as soon as the injection mass reached the brain.

In no instance was a complete injection obtained and there were variations from animal to animal. Perhaps the most constant result was the injection of the cervical lymph nodes. At one time or another many of the peripheral nerves were injected. Specifically there may be mentioned the brachial plexus and its branches as far distally as the hand; the 2 sympathetic chains and their ganglia; the phrenic nerves including their ramifications on the diaphragm; the intercostal nerves and their branches. The position of the ligatures for the cannulae prevented the injection of the nerves of the caudal extremities.

[1] Funaoka, S., und Yamada, J., *Folia Anatomica Japonica*, 1929, **7**, 399.

Other injection masses have been found satisfactory. The important factor seems to be to select one that is not readily miscible with the body fluids.

6422

A Method for Making Quantitative Intestinal Studies.

CHARLES G. JOHNSTON. (Introduced by S. Goldschmidt.)

From the Laboratory of Surgical Research, University of Pennsylvania.

The intestinal loop devised by Thiry[1] and modified by Vella[2] has been used by numerous workers in studying the absorption of various materials from the intestine. Either the original method or its modification permitted observation of qualitative changes in the introduced material in an unanesthetized animal. Furthermore, the loop is more or less permanent and permits repeated experiments on the same animal under similar and varying conditions.

The Thiry loop has one fistulous opening on the anterior abdominal wall while the Vella modification consists of a fistulous opening at each end of the intestinal loop. The chief objection to either of these methods is the inability to prevent leakage. In an attempt to overcome this difficulty Gumilewski[3] placed rubber balloons in both ends of a Thiry-Vella loop. One balloon served as a plug while the other was penetrated by a short rubber tube which permitted filling and emptying of the loop. Modifications of the Gumilewski method have been used by Nagano,[4] Cobet,[5] and White and Rabinowitch.[6] The Gumilewski technique has 2 definite disadvantages. A single balloon placed within the loop has a tendency to be drawn in or pushed out by peristalsis, and it is difficult to anchor the balloons sufficiently to make an effective seal. With a short tube in the end of the loop the fluid tends to pocket in segments making complete emptying almost impossible. Loops prepared by the old Thiry technique, in which the gut is brought through the abdominal wall, have a tendency to prolapse through the fistulous opening. In order to obviate certain of the difficulties

[1] Thiry, *Sitzgsber. d. Wiener Acad. Math.-naturhist.* Abth. L. 1864, 77.

[2] Vella, S., *Moleschotte unters, Z. Naturlehre,* 1882, **13**, 40.

[3] Gumilewski, D., *Arch. Pflüger,* 1886, **39-40**, 556.

[4] Nagano, J., *Arch. Pflüger,* 1902, **90**, 389.

[5] Cobet, R., *Biochemische Zeitschrift,* 1921, **114**, 33.

[6] White, H. L., and Rabinowitch, J., *J. Biol. Chem.,* 1927, **74**, 449.

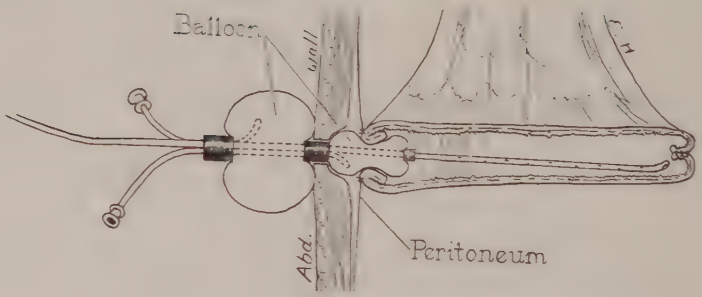


FIG. 1.

of the older methods we have modified the technique both from the standpoint of the method of making the loop and of sealing it for studies of a quantitative character.

Dogs have been used in all of our work, but the method is suitable for any animal where loop studies are desired. The initial part of the operation consists essentially of a Thiry fistula. The length has varied from 20 to 50 cm. After the continuity of the bowel has been reestablished by end-to-end anastomosis, the distal end of the loop segment is closed by a continuous suture and the stump inverted and oversewed by an interrupted seromuscular suture. The proximal end of the loop is then carefully inverted around a fairly large rubber tube which extends partly into the loop. The inversion is obtained by 2 purse string sutures so that approximately 3 cm. of the proximal end of the loop are inverted. Interrupted sutures of silk are placed at 4 equidistant points on the loop and the ends are left long. At a convenient point on the abdominal wall a small stab wound is made and the free end of the rubber tube is brought through this. When this is done the loop is snug against the anterior parietal peritoneum so that visceral and parietal peritoneum are adjacent. The double ends of the 4 sutures attached to the proximal end of the loop are then threaded on a straight skin needle and passed through the thickness of the abdominal wall in such a manner as to emerge about a centimeter from the tube. One end of each pair of sutures is passed through a piece of rubber tubing and the 2 ends tied so that there is no tendency for the sutures to cut through the tissues. When this is completed a piece of omentum is wrapped around the point where the loop is attached to the abdominal wall and held with 2 or 3 interrupted sutures. The abdominal wall is then closed. No dressing is placed over the tube, so that any material accumulating in the loop may have easy egress and the animal is not soiled. Every 24 hours the loop is washed

out with warm normal saline solution. For about one week the animal is kept on a very smooth diet, being fed frequently but in small amounts. After this period he is put on the routine animal house diet. Within 2 weeks the rubber tube is so loose that it can be easily withdrawn.

Loops prepared by this method do not intussusept. A soft Nelaton catheter is inserted daily along the fistulous tract and the loop is gently irrigated. The loop is ready for study, as a rule, in about 2 weeks from the time of the operation. The length of the loop is measured at the operation so that when the animal is ready for study it only remains to provide it with a catheter, fitted with balloons which will prevent leakage; that will completely empty the loop. For this purpose we have again modified existing techniques.

A No. 14 French soft rubber catheter is perforated freely along that portion which is to lie within the loop of intestine. Along the side of this catheter are cemented 2 short No. 8 catheters, one ending at a point about 1.5 cm. distal to the place where the catheter meets the skin and the second one terminating at a similar point proximal to this same place. Small balloons made from the fingers of a latex glove are placed around the main catheter at the points where the 2 smaller catheters terminate.

The catheter with the balloons deflated is inserted into the intestinal loop so that the lower balloon lies just within the loop and the upper one lies partly within the fistulous tract in the abdominal wall and is partly exposed on the abdominal wall. The inner balloon is then inflated with a small amount of air, an amount just sufficient to allow it to be pulled easily into the fistulous tract but not out of it. The outer balloon is then inflated so that the balloon lies wholly upon the abdominal wall. As the outer balloon is inflated it draws out of the fistulous tract completely, pulling the partially inflated inner balloon into the opening in the abdominal wall. The buried balloon snugly closes the proximal end of the loop but does not cause distention of it. The proximal balloon when distended prevents peristaltic action from pulling the catheter into the loop. If the catheter has been made properly, one can introduce fluids and recover them quantitatively with an accuracy closely approximating 100%. Each animal has its own set of catheters which are examined daily to make sure that they are in perfect shape.

The investigator is naturally concerned as to whether or not loops of this type function as normal intestinal membranes over a considerable period of time. If Nagano[4] was correct when he stated that intestinal loops very easily become the seat of "catarrhal in-

flammation" some evidence of this should be easily obtained. Examination of Nagano's data will disclose that on the basis of it the statement is not correct. Subsequent to the period at which his data were interpreted as the result of catarrhal inflammation he, in each instance, obtained data nearly identical with the data obtained from a loop at a time when he considered the membrane normal.

We have demonstrated normal peristalsis in loops prepared as described as long as 7 months after their preparation. At this period sodium chloride and glucose, in various concentrations, were being absorbed at a rate which was within the limits of error of the rates of absorption of these substances in the same animal some months previously. The rate of secretion from a loop does not increase with time. There is a considerable variation in the rate of secretion in different intestinal levels as there is at the same level during various periods of the day, but there is no evidence of a weekly or monthly variation when taken as a whole. Furthermore, histologic examination of the loop wall even months after preparation of the loop discloses no microscopic change in any of its structures.

These are criteria upon which one should be able to obtain some evidence of the change in the wall as a membrane. We have failed to find any evidence which would lead us to believe that these intestinal loops are not normal and that this condition does not continue over many months with ordinary care. Secretion from the loops 7 months after their preparation discloses enzyme activity.

6423

Note on the Failure of Anterior Lobe Extract to Pass from Fetus to Mother.*

G. B. WISLOCKI AND F. F. SNYDER.

From the Departments of Anatomy and Obstetrics, Johns Hopkins University.

In a previous paper[1] we showed that ovulation can be induced in the pregnant rabbit by intravenous injection of concentrated human urine of pregnancy or anterior lobe extract of beef.† This observation was the basis of further experiments here reported.

* This research was aided by a grant from the Linton Fund.

[1] Snyder, F. F., and Wislocki, G. B., *Johns Hopkins Hosp. Bull.*, 1931, **49**, 103.

† The concentrated urine of pregnancy referred to is the so-called "luteinizing" hormone prepared by Parke, Davis & Co. The extract used was stated to contain 50 rat units per cc. The experiments with anterior lobe were carried

Since a pregnant rabbit could be made to ovulate by injecting either of these substances, it appeared that this phenomenon might well be used to investigate the possibility of the passage of anterior lobe substance from fetus to mother. We used 6 pregnant rabbits. The rabbits were opened under ether anesthesia by laparotomy. The injections were made through the unopened uterus directly into the fetuses by the aid of a syringe and a fine gauge needle. In all instances the material was injected into the peritoneal cavity or the neighboring musculature of the rump of the fetus. Into a fetus in one rabbit we injected 1 cc. of concentrated human urine of pregnancy. Into the fetuses in the other 5 rabbits various amounts from 2 to 8 cc. of anterior lobe extract (beef) were injected. Two days later the mother was opened again under anesthesia, so that the ovaries could be examined. At the same time the fetuses were observed to see that they were still living and their circulation intact. In the observations reported, all of the fetuses were alive at the termination of the experiments, so that failure of the anterior lobe extract to cause ovulation in the mothers can not be attributed to fetal death.

TABLE I.

Effect on ovaries of pregnant rabbits of injecting concentrated human urine of pregnancy (P. D. & Co.) or anterior lobe extract (P. D. & Co.) into the fetuses.

No.	Day of pregnancy	Amt., mode of administration	Examination of ovaries of mother 2 days later
1	28	1 cc. conc. urine of pregnancy into 1 fetus	No ovulation
2	29	2 cc. ant. lobe extr. into 1 fetus	" "
3	26	2 cc. ant. lobe extr. into 1 fetus	" "
4	26	6 cc. ant. lobe extr. into 3 fetuses	" "
5	26	6 cc. ant. lobe extr. into 3 fetuses	" "
6	26	8 cc. ant. lobe extr. into 4 fetuses	" "

The results are shown in Table I. In no instance did ovulation occur in the mother after injection of anterior lobe extract into the fetuses. The series is small, but in 3 instances relatively large amounts of anterior lobe extract were injected into the fetuses. Although we have not investigated the minimum dosage of this substance required to make a pregnant animal ovulate, our previous experiments show that 2 cc. of anterior lobe extract injected into the blood stream of the mother during the last half of pregnancy induces ovulation. The effectiveness of our preparation of anterior

out with the aid of anterior lobe extract of the hypophysis (beef) prepared by Dr. Bugbee of Parke, Davis & Co. More detailed information concerning these products is to be found in our previous paper.

lobe extract was controlled by injecting 2 cc. of the same extract intravenously into animal 4 and 1 cc. into animal 2 four days after the failure of the injection into the fetuses to produce ovulation. Following each of these, subsequent injections of the extract directly into the mother, ovulation occurred.

Apparently the tissues of the fetus or the placental barrier withheld the passage from fetus to mother of an amount of the substance sufficient to produce ovulation. These results indicate that anterior lobe extract is probably not transmitted from fetus to mother. It is possible, however, that with even greater dosage, or by direct introduction of the extract into the fetal blood stream, the substance might be induced to pass the placental barrier in sufficient amount to cause ovulation. Nevertheless the failure of anterior lobe extract to pass the placenta in the present experiments and to exhibit a biological effect in the mother is in keeping with observations on the failure of transmission of active principles of other glands (adrenalin, insulin, pituitrin, and parathyrin) (Snyder and Hoskins,[2] Hoskins and Snyder.[3])

6424

A New Symptom Complex in Vitamin-G Deficiency in Rats.

SUSAN GOWER SMITH. (Introduced by H. L. Amoss.)

From the Department of Biochemistry, Duke University School of Medicine, Durham, North Carolina.

The diversity of symptoms resulting from vitamin-G deficiency and the lack of uniformity of results even in the same laboratory lead to confusion in evaluating the real significance of any one symptom. The present report deals with a new symptom which thus far has been found to occur regularly.

Some of the symptoms of rats on vitamin G deficient diets described most frequently by other investigators are dermatitis occurring at various sites on the body,[1] a peculiar oedematous dermatitis of the digits,[3] alopecia,[2] blood stains on wrist and forepaws,[3] and

[2] Snyder, F. F., and Hoskins, F. Meredith, *Anat. Rec.*, 1927, **35**, 23.

[3] Hoskins, F. M., and Snyder, F. F., PROC. SOC. EXP. BIOL. AND MED., 1927, **25**, 264.

[1] Goldberger, J., and Lillie, R. D., *Pub. Health Rep.*, 1926, **41**, 1025.

[2] Chick, H., and Roscoe, M. H., *Biochem. J.*, 1927, **21**, 698.

[3] Chick, H., and Roscoe, M. H., *Biochem. J.*, 1928, **22**, 790.

dark reddish brown stains,[4] which fail to give the tests for blood, on the lower abdomen, and about the urethra, chromogenic urine and constipation. In the present study all of these symptoms occurred at some time during the course of the experiment in different individuals. The most common one was the blood stained wrists and forepaws but this was later shown to be a dehydration phenomenon, occurring regularly in rats deprived of water.

Eighteen white albino rats of the Wistar Institute stock were placed on Sherman's vitamin G deficient diet No. 554[5] at the age of 45 days, for a period of 226 days. The 17 rats, surviving longer than 70 days, all developed a characteristic dermatitis of the tail which manifested itself grossly by a coating of brownish yellow waxy material which grew progressively worse until the animal died or had its diet supplemented with autoclaved yeast. Upon the addition of yeast, autoclaved for 2½ hours at 15 pounds pressure, this condition gradually cleared up.

The material coated on the tails failed repeatedly to give a positive benzidine or guaiac test for blood. It did, however, give a positive test for lipoid material when dissolved in ether and stained with Sudan IV.

Microscopic sections* made from the tails of all rats having this dermatitis showed very characteristic histological changes in the skin (Fig. 1). Although the epithelium remained intact it showed thinning and disorganization. There was almost complete atrophy and disintegration of the sebaceous glands. In addition there was atrophy and fragmentation of the fibrillar material in the corium.[6] There was no cellular infiltration. These histological changes preceded the gross changes by an appreciable period of time. The one rat dying at the end of 70 days on the diet showed no gross lesion, but microscopic sections of the tail showed characteristic histological changes described above.

If after 226 days on the deficient diet, a supplement of autoclaved yeast, at a level of 10%, is added for 36 days and the tails then biopsied and sectioned the histological picture is entirely changed. The epithelium is completely regenerated and the sebaceous glands and corium have returned to normal.

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[4] Leader, K. R., *Biochem. J.*, 1930, **24**, 1172.

[5] Sherman, H. C., and Bourquin, A., *J. Am. Chem. Soc.*, 1931, **53**, 3501.

* I am indebted to Dr. D. H. Sprunt of the Department of Pathology for examining the microscopic preparations and confirming statements made about them and to Miss Eleanor Milnor for technical assistance.

[6] Denton, Jas., *Am. J. Path.*, 1928, **4**, 341.

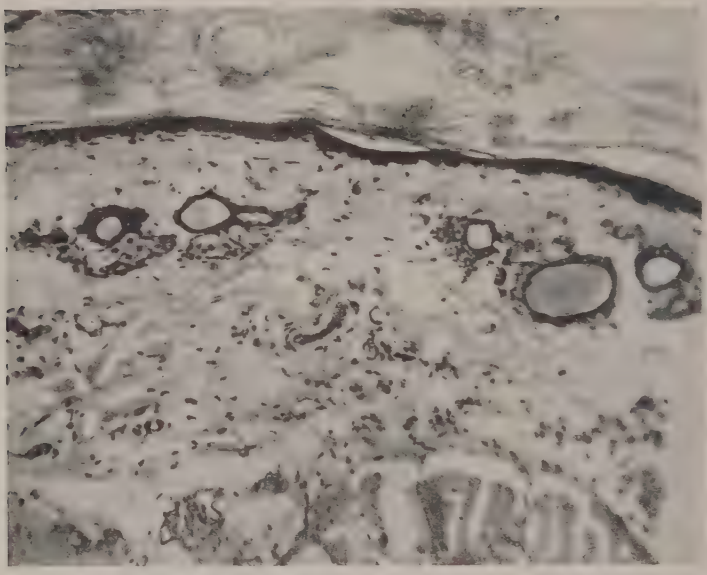

FIG. 1.
Tail of Rat No. 36 after subsisting on G-deficient diet No. 554 for 226 days. Tail at this time exhibited the gross lesion described in text.

The same symptom complex was observed in 17 rats on a diet similar to one on which human beings frequently develop pellagra. This diet consisted of white, water-ground, corn meal 50%; pork fat 20%; cane syrup 15%; white flour 10%; and cane sugar 5%.

In order to rule out the effects of dehydration or inanition, 24 rats were subjected to 2 starvation regimes. In 10 rats deprived of food but given water there occurred merely loss of weight and general weakness but no characteristic lesion. Twelve rats deprived of water but given food developed the blood stained wrists often described as a symptom in vitamin G deficiency. The mouth, nose, and eyes were also stained. However, not a single individual in these 2 groups developed the tail lesions described above, either grossly or microscopically.

Sections made from the tails of 16 control rats were all entirely normal.

These experiments are being repeated and tests made to determine whether the microscopic changes might be used in developing a new assay method for vitamin G.

6425

Carotene and Vitamin A.

HARRY GOLDBLATT AND HAROLD M. BARNETT.

From the Institute of Pathology, Western Reserve University.

Carotene was adopted as a temporary international standard for Vitamin A potency by the Permanent Standards Commission of the Health Organization of the League of Nations. Since then much interest has been shown in an exact determination of the value of this unit in terms of the Sherman Unit. The earlier literature assigns values between 2 λ and 20 λ of carotene as meeting the requirements for one Sherman Unit, while the more recent results of Polak and Stokvis[1] give values as low as 0.5 λ of carotene to one Sherman Unit. This paper is offered as a further contribution to this subject.

In preparing carotene from carrots by a modification of the method given by Schertz,[2] part of the carotene extracted was obtained in crystalline form and part remained in the concentrated petroleum ether extract. After removal of the petroleum ether by evaporation under proper conditions, a deep red carrot oil remained. Moore[3] found carrot oil potent as a source of Vitamin A. However, his work was not quantitative on the basis of the content of pigment. Hence, the questions arose, whether or not the pigment which remained in the carrot oil was as potent a source of Vitamin A as that which crystallized, and whether there was any growth-promoting factor in carrot oil other than that due to its carotene content when all the color in the carrot oil is considered as carotene. To determine this biologically, 2 test solutions were prepared as follows:

Solution 1. Crystalline Carotene Dissolved in Wesson Oil: A sample of carotene isolated from carrots was found to have a purity of 79% when tested by a modification of the potassium dichromate comparison method of Palmer,[4] based on the earlier results of Willstätter and Stoll. 5.1 mg. of this carotene were dissolved in 100 cc. of Wesson Oil, thus giving the oil an actual carotene content

[1] Polak, A., and Stokvis, J. A., *Arch. neerlandais de physiol. de l'homme et des animaux*, 1931, **16**, 542.

[2] Schertz, F. M., *J. Agr. Research*, 1925, **30**, 469.

[3] Moore, T., *Biochem. J.*, 1929, **23**, 803.

[4] Palmer, L. S., Carotinoids and Related Pigments, Chemical Catalog Co., Inc., New York, 1922, 259.

of 0.001 mg. (1 λ) in each drop from a pipette standardized to deliver 40 drops per cc.

Solution 2. Carrot Oil Diluted with Wesson Oil: Carrot oil prepared by the method outlined above was tested by diluting samples with petroleum ether and comparing with 0.2% $K_2Cr_2O_7$ solution. It was found to have color equivalent to 2.72% carotene.* The carrot oil was diluted 9:1 with Wesson Oil and when this was diluted with petroleum ether it gave a color equivalent to 0.27% carotene. 1.5 g. of this diluted carrot oil was further diluted to 100 cc. with Wesson Oil, thus giving a carotene content, as determined colorimetrically, equal to that of Solution 1, that is, 1 λ per drop (0.025 cc.) of oil. The original color of these 2 dilute solutions of carotene was retained throughout the complete biological test period.

Biological Tests. Albino rats† about 4 weeks old and weighing about 45 gm. were fed on a diet deficient in Vitamin A until they had definitely ceased gaining weight. This period varied from 6 to 9 weeks. The diet was composed as follows: Casein (inactivated) 20 gm., irradiated olive oil‡ (for Vitamin D) 15 gm., cornstarch (free of Vitamin A) 50 gm., salt mixture (McCollum, No. 185) 5 gm., yeast (Harris Medicinal) 10 gm. This was served as a very thick paste made by adding 50 cc. of water.

After the rats had definitely ceased gaining weight for about 2 weeks daily supplements of varying quantities of solutions 1 and 2, described above, were administered orally, by pipette, standardized to deliver 0.025 cc. per drop. Solutions 1 and 2 were further diluted with Wesson Oil in order to permit the administration of smaller quantities of carotene. Tables I and II give the average gains per week for the first 4 weeks and for the 8 weeks following the beginning of the administration of the solutions. The average gain for the first 4 weeks is also given in the tables because this is the method used by some of the British investigators. The average gain during

* In making colorimetric determinations of this type it is important to have the oil so diluted that at least 98% of the solvent is petroleum ether. Otherwise a correction should be made, as the color intensity of carotene in oil is several times that of an equal quantity dissolved in petroleum ether and the test is based on the color intensity of carotene in petroleum ether. This point has not been generally recognized, and it is planned to make it the subject of a later communication.

† Wistar strain, bred at the Institute of Pathology.

‡ 300 cc. olive oil in an open flat dish 35 cm. square irradiated for ½ hour at a distance of 40 cm. from the burner of a Cooper-Hewitt mercury vapor quartz lamp.

TABLE I.
Solution 1. Each rat given crystalline carotene dissolved in Wesson oil.§

Amt. carotene (mg.)	Aver. gain per week during first 4 wks. (gm.)	Aver. gain per week during 8 weeks (gm.)
.00025	Lost	Died in 4 weeks
.00025	"	" " 3 "
.00025	"	" " 3 "
.0005	3.5	2.8
.0005	5.0	2.9
.00075	6.2	3.6
.001	5.8	4.6
.001	9.0	5.8
.002	16.3	9.6
.002	8.5	6.6
.002	8.6	7.0
.003	10.0	7.0
.003	10.5	8.1
.004	16.7	8.3
.004	9.2	7.5
.008	17.7	14.0

§Control rats that received daily 8 drops of Wesson oil alone, lost weight and died in from 3 to 6 weeks after the end of the depletion period.

TABLE II.
Solution 2. Carrot oil diluted with Wesson oil.

Amt. carotene (mg.)	Aver. gain per week during first 4 wks. (gm.)	Aver. gain per week during 8 weeks (gm.)
.00025	Lost weight	Lived 4 weeks
.0005	6.2	3.9
.0005	5.1	3.8
.00075	6.6	4.0
.001	11.2	6.2
.001	8.0	5.6
.001	6.5	5.0
.002	10.2	6.0
.002	8.0	5.8
.003	13.2	6.5
.004	11.7	6.5
.004	13.5	9.7
.008	16.0	10.6

the 4 weeks was always greater than during the 8 weeks. The results show that of each solution a quantity containing 0.0005 mg. (0.5 λ) of carotene was the minimum that just satisfied the requirements for a Sherman unit of Vitamin A, that is, an average gain of 3 gm. per week for 8 weeks. The number of animals on the lower doses was small because the previous work of other investigators did not lead us to expect the potency found in the solutions here described.

The results show that the color in freshly prepared carrot oil, when considered as carotene, is equal in Vitamin A potency to an equivalent amount of crystalline carotene. This not only confirms the opinion that carotene is the only pigment of consequence in

carrot oil, but also indicates first, that carotene is probably the only growth-promoting factor of the order of Vitamin A in carrots, and second, that the potency of carotene is not increased by the presence of any of the other constituents of carrot oil.

The results also confirm those reported by Polak and Stokvis,[3] who found carotene to be much more potent as a source of Vitamin A than had previously been reported by other investigators. Should it become generally recognized that 0.5 λ of carotene satisfies the requirements for a Sherman Unit of Vitamin A, then one International Unit of Vitamin A (1 λ of pure carotene) may be considered as equivalent to 2 Sherman Units.

The extreme variation (0.5 to 20.0 λ) in the amount of carotene required for one Sherman Unit of Vitamin A, as reported by various investigators, is not in every case readily explainable. In some instances the lower potencies may have been due to factors such as the use of impure carotene preparations, loss of carotene by oxidation during the preparation of the solution or during the biological tests, or to methods of administration which resulted in faulty assimilation. In the tests here reported precautions were taken against these 3 factors. The solutions were made up on a basis of the actual carotene content as determined colorimetrically; periodic tests showed that the original color of the 2 dilute carotene solutions was retained throughout the biological test period; and, to our knowledge, the most desirable method of administration was used. In some preliminary tests we found that solutions of carotene in ethyl laurate and in olive oil aerated at 120° C. for 6 hours became completely decolorized in a few weeks. Similar results have been reported by others. It is possible that there was more or less loss of carotene in this way in some of the cases reported where higher doses of carotene were required to promote growth. Further research may also show considerable variation in the percentage of carotene assimilated depending upon the type of solvent and method of administration.

6426

"Action Currents" Without Rhythmic Contractions and Rhythmic Contractions Without "Action Currents."

JOSEPH BERKSON,* EDWARD J. BALDES AND WALTER C. ALVAREZ.

From the Divisions of Medicine, The Mayo Clinic, and Division of Physics and Biophysical Research, The Mayo Foundation, Rochester, Minnesota.

The present-day teaching is that action currents obtained from contracting muscle are due to physico-chemical changes associated with the shortening of the fibres. Against this view is the fact that electrocardiograms have been obtained during experiments in which the heart-beat was, to all appearances stopped, by the removal of calcium from the nutrient fluid or by other means. Similarly, we have been able to show that the characteristic electrogram that is associated with the rhythmic contractions of the bowel persists long after the muscular movements have been stopped by epinephrine.

Rabbits were used anesthetized with urethane and with the abdomen open in a bath of physiologic saline solution. The loops of bowel being studied were kept in a layer of mineral oil, which floated on the surface of the salt solution. The electrodes consisted of steel wire serrefines plated with silver and silver chloride, and so suspended on pivots that the movements of the bowel could be recorded simultaneously with the electrogram. A string galvanometer was used. Details of the technic will be published in a paper now in press.

In a typical experiment with epinephrine 0.15 cc. of a 1 to 1000 solution was injected into the ear vein. Within 10 seconds the contractions of the bowel disappeared while the electrical changes persisted. The only modification in the electrical record consisted of a greater regularity of the waves and a more rapid rate. In one experiment the increase was from 9 to 12 cycles a minute. After 105 seconds the mechanical contractions returned in the lower part of the bowel and the electrical variations were then found to be in phase with them.

In an attempt to explain this phenomenon we were at first inclined to assume that something had happened to one of the links in the chain of chemical processes that bring about contraction in the muscle fibers; to use a metaphor derived from the field of me-

* Fellow in Medicine, the Josiah Macy, Jr., Foundation, on duty in The Mayo Foundation.

chanics, the engine continued to run after a clutch of some kind was thrown out. But what sort of a theory could we evolve to explain matters when, after the administration of nicotin, we found that the rhythmic contractions continued unchanged and the electrogram disappeared?

In a typical experiment a short segment of rabbit's intestine was excised and allowed to contract rhythmically in a layer of mineral oil floating on top of warm Ringer-Locke's solution. Simultaneous records were obtained of the mechanical and electrical changes. One-half cubic centimeter of a 1 to 100 solution of nicotin was added to the 80 cc. of Ringer's solution in the bath. Immediately there was a marked disturbance in both records. The muscle contracted down and remained that way while the electrical record showed violent erratic changes. During the next 4 minutes the electrical record consisted of a series of small regular waves, interrupted by erratic variations. Many of the cycles were miniatures of the usual ones obtained during the course of normal rhythmic contractions. After 5 minutes the regular rhythmic contractions of the muscle returned while the electrical record showed erratic changes with very small amplitude. After 14 minutes the mechanical waves were vigorous, the amplitude was larger than normal, and the rate was slowed to 6 a minute. The electrical changes had almost disappeared. After 77 minutes the mechanical contractions were regular and vigorous while the electrical changes had entirely disappeared. At the end of 3 hours the rhythmic contractions became feeble and irregular and after 5 hours they stopped. At no time was there a return of electrical changes.

6427

Active Immunization Against Tunisian Typhus Fever with Mexican Typhus Vaccine.*

GERARDO VARELA, ANGEL PARADA AND VIRGILIO RAMOS.
(Introduced by Hans Zinsser.)

From the Hygienic Institute of Mexico.

Several attempts have been made to vaccinate human beings against typhus fever, both with living virus[1] and with killed rick-

* We wish to express our thanks to Doctors Manuel Madrazo and Ruiz Castaneda for their advice and help in the elaboration of the vaccine.

[1] Nicolle, C., and Conseil, E., *Compt. Rend. Acad.*, 1910, **151**, 598.

ettsia. Immunization with living virus is always dangerous, since it is extremely difficult to make accurate titrations of the virulent material, so any method yielding a rich suspension of killed rickettsia, safe to handle and at the same time producing an effective immunity would be a great help in typhus fever epidemics.

Weigl[2] obtained encouraging active immunizations by means of an emulsion of the triturated intestinal canals of infected lice, suspended in 0.5% phenolized salt solution. His vaccine corresponds to 100 lice per cc. and is administered in 3 weekly doses. In our experience this vaccine produces in man a rather severe reaction similar to that of typhoid vaccine. Although there is no doubt as to the effectiveness of the Weigl suspension, its manufacture on a large scale is not practical.

Following the discovery of Mooser,[3] who found, in the *tunica vaginalis* of guinea pigs infected with Mexican typhus, a small intracellular organism that by its characteristics has been identified with the rickettsia of the louse, Zinsser and Castaneda have attempted[4] to produce large amounts of these organisms which, killed in a convenient manner, would give suitable material for active typhus immunization. They succeeded in this by infecting rats previously treated with benzol or exposed to X-ray radiation. These rats present a massive infection of the peritoneal cavity, smears from which show an enormous number of intra- and extracellular rickettsia, giving the appearance of a culture. The peritoneal washings made with formalinized salt solution give a fairly strong vaccine.

In the following experiments we used a vaccine prepared by means of the benzol method, though actually we now prepare the vaccine by the recent X-ray technique published by Zinsser and Castaneda[5] which results in a suspension of rickettsia bodies as free as possible from rat serum and cells.

Procedure. Adult male rats are exposed to an X-ray radiation of 170 KV constant potential; 80 cm. distance; 0.5 mm. copper filter plus 4 mm. celluloid; 8 milliamperes; the effective wave length 0.160 Angstrom units; intensity 10 "r" units per minute; 1 hour exposure (600 "r" units). Immediately after radiation, the rats are intraperitoneally inoculated with an emulsion made with tunica from a Mexican typhus-infected guinea pig. The rats appear sick

[2] Weigl, *Med. Klinik.*, 1924, 1046.

[3] Mooser, H., *J. Infect. Dis.*, 1928, **43**, 261.

[4] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1930, **52**, 649.

[5] Zinsser, H., and Castaneda, M. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 840.

on the 3rd day after inoculation and on the 5th day are killed and thoroughly bled. With a specially made pipette of about 10 cc. worked with a rubber bulb, the peritoneum and intestines are carefully washed with some 100 cc. of a 0.2% formalinized salt solution. The washings are centrifuged for 30 minutes at low speed and the supernatant is decanted for further treatment. The sediment is carefully ground with pyrex glass in a mortar and successive centrifugations and washings are worked out in order to break up the cells and obtain as many free rickettsia as possible. The supernatant is added to the first material and submitted to rapid centrifugation (5000 r.p.m.) for an hour. The new supernatant is discarded and the sediment broken up with a pipette. By repeated low-speed centrifugations and washings, this sediment will give a suspension of rickettsia which appear to be free from cells. A centrifugation at high speed will give a sediment which, resuspended as before in formalinized salt solution, will give a vaccine as free as possible from rat protein. This suspension is then standardized so as to give a count of 1000 million rickettsia per cc. For use, we prepare 3 increasing doses of 500 to 1000 million, to be injected at weekly intervals.†

For our experiments we used a Tunisian strain of typhus which was kindly given us by Professor Nicolle. Our first step was to investigate the dose of guinea pig brain sufficient to infect 100% of the guinea pigs and yet not so strong as to impair the immunity produced by the vaccination. Sixteen guinea pigs were inoculated with suspensions of brain varying from 1/50 to 1/10,000 in salt solution. All animals receiving dilutions up to 1/5,000 gave typical typhus. When the dilution was as high as 1/10,000, a longer incubation time (12 days) was observed. We decided to use a dose of 1/500 for test in our vaccinated guinea pigs, an amount which would certainly infect our normal controls. Twenty guinea pigs were observed for several days and found satisfactorily healthy. Ten were injected with 0.5 cc. of the formalinized Mexican vaccine. Seven days later, the same animals received another dose of 0.5 cc.; and after another week a final dose of 1.5 cc. Nine days after the last dose of vaccine, the 20 guinea pigs were intraperitoneally inoculated with a

† In the form in which this vaccine is delivered, the suspensions are in such physical condition that it is expected that a lasting antigenic power will be obtained which seems superior to that in the raw vaccine (Kemp)—perhaps on account of the considerable amount of protein—in which the intracellular rickettsia are probably fixed in the protoplasm by the formalin, thus mechanically diminishing its antigenic activity.

1/500 suspension of the brain of a Tunisian-infected guinea pig. Temperatures were taken twice a day during the vaccination without any considerable rise being noted. This is contrary to the observation of Zinsser and Castaneda, but was probably due to the small amount of vaccine used in our experiment as compared to the doses used by them. All the controls gave typical reactions and of the 10 vaccinated animals only 5 had fever. The others proved to have been completely protected against the infection.

We find it interesting to publish these experiments, even though they are not extensive, because they prove that even with a small amount of vaccine it is possible to protect guinea pigs against the Old World strain by the Mexican vaccine of Zinsser and Castaneda.

Encouraged by the recent work of Sanchez Casco[6] we have vaccinated 2153 persons, mainly among employees of the public bathing houses for indigents and among the inmates, physicians and nurses of the Belen prison, where a focus of typhus was recently discovered. In no case did we observe any accident due to the vaccination. Dr. M. Bustamante of the Department of Transmissible Diseases has vaccinated some 6,000 persons in epidemic zones, and every day the demand and use of the vaccine is greater. We feel confident that this method of protection will prove of great assistance in the typhus problem in this country.

6428

On Individual Differences in Chicken Blood.

K. LANDSTEINER AND PH. LEVINE.

From the Laboratories of the Rockefeller Institute for Medical Research, New York.

I. The existence of numerous individual serological differences in the blood of chickens has been established by several authors by means of rabbit immune agglutinins,[1] immune isoantibodies,[2] and normal isoantibodies.[3, 4] The most interesting and exhaustive

[6] Sanchez-Casco, R., *Medicina*, 1932, **12**, 316.

[1] Landsteiner, K., and Miller, Jr., C. P., PROC. SOC. EXP. BIOL. AND MED., 1924, **22**, 100.

[2] Todd, C., *Proc. Roy. Soc.* B., 1930, **106**, 20, **107**, 197; see Hadda, S., and Rosenthal, F., *Z. Immunitätsforsch.*, 1912-1913, **16**, 524.

[3] Karshner, W. M., *J. Lab. Clin. Med.*, 1928-1929, **14**, 346.

[4] Shimidzu, T., *Tohoku J. Exp. Med.*, 1931, **18**, 97; see Bailey, C. E., *Am. J. Hyg.*, 1923, **3**, 370.

studies were carried out by Todd, who demonstrated with the use of immune isoagglutinins an almost complete individual specificity —a result in harmony with his older experiments with White[5] on cattle blood.

As is seen from the following tests, a differentiation of individual chicken blood can also be made without difficulty by using absorbed normal ox sera.

TABLE I.

Absorptions were made by mixing inactivated ox serum diluted 1:2 with ½ its volume of washed and packed chicken blood cells. After one hour at room temperature, the mixtures were centrifuged. Tests were made by adding 2 drops of the fluid to 1 drop of a 2.5% suspension of washed chicken blood cells. Readings after 2 hours at room temperature.

Three absorptions were made with bloods 4, 7, and 10. With all the other bloods, 2 absorptions were made, although the fluids were completely exhausted after one treatment.

Absorbed with cell No.	Chicken Cells No. 1	2	3	4	5	6	7	8	9	10	11	12
1	0	+	±	++	+±	+±	++	±	+	++±	+	0
2	0	0	±	++	+±	+	++	tr	±	++±	+	0
3	0	±	0	++	±	0	++	0	tr	++±	tr	0
4	0	±	0	±	0	0	+±	0	tr	++±	0	0
5	0	0	0	+±	0	0	++	0	ftr	+±	0	0
6	0	±	0	++	±	0	++	0	±	++±	tr	0
7	0	0	0	0	0	0	0	0	0	0	0	0
8	0	±	0	+±	±	ftr	++±	0	tr	++±	ftr	0
9	0	±	0	++	±	tr	++	0	0	++±	0	0
10	0	0	0	0	0	0	ftr	0	0	0	0	0
11	0	+	0	++	+	±	++±	ftr	±	++±	0	0
12	0	+±	+	++	+±	++	++±	+±	+	++±	+	0

The intensity of the reactions is indicated as follows: 0, ftr (faint trace), tr (trace), ±, ±, +, ++, etc.

The tests (Table I) show that the agglutinins present in normal serum permit of a differentiation of individual blood properties, since most of the 12 chicken bloods examined could be distinguished from each other. From some preliminary experiments it seems possible that the differentiation can be carried further by using various normal sera.

As these findings suggested similar experiments with the blood of other species, tests were made with guinea-fowl and turkeys. Within these species as well, certain differences were noticed, but the variations were much less pronounced. If one would venture to offer an explanation for these facts, it might be suggested that possibly the greater diversity in the blood of chickens is correlated to the existence of a large number of somatic variations in this species, re-

[5] Todd, C., and White, R. G., *Proc. Roy. Soc.*, B, 1910, **82**, 416; *J. Hyg.*, 1910, **10**, 185; *Proc. Roy. Soc.*, B, 1911, **84**, 255.

sulting from extensive selective breeding and perhaps the derivation from several ancestral forms.

II. A striking aspect of the experiments of Todd and White on cattle and of Todd on chicken blood is, as already mentioned, the large number of differences. This great diversity would seem less puzzling if it could be explained by the existence of more or less well defined serological factors, such as exist in human blood.[6, 7] Thus the combination of n independent factors would yield 2^n differences. Actually in cattle blood a division into groups has been demonstrated by Little, and by Hofferber and Winter, and from Todd's genetic studies on chicken families, certain types appeared to exist. Furthermore, in experiments made by L. C. Dunn and one of the writers, an agglutinogen, whose heredity was apparently that of a single dominant property, was found in several chicken families by means of an anti-chicken rabbit immune serum.

In the following experiments (Table II), 2 types of chicken blood could be demonstrated with the aid of normal ox sera and Forssman immune sera, produced by injecting rabbits with horse kidney, or alcoholic extracts of cat or dog blood along with pig serum. On testing batches of chicken bloods with the diluted ox sera, it was seen that practically all gave the strongest agglutination reactions with the same bloods whereas others were consistently weakly agglutinable. Also when the cells were tested with various Forssman immune sera, it was observed that all acted similarly in that the strongest agglutination took place with the same cells which were, however, not those most sensitive to the ox sera. This specificity is seen from Table II, representing tests with 9 out of about 30 chicken bloods, those having been selected which reacted most intensely with

TABLE II.

The ox sera were diluted 1:20, the Forssman antisera 1:10. Tests and readings were made as in the experiments recorded in Table I.

Sera	Chicken Cells No. 7	10	4	15	16	8	11	17	18
Ox No. 1	++±	++±	++	++±	0	0	±	±	0
Ox No. 2	++±	++±	++±	++±	0	0	±	+	0
Ox No. 3	++±	+++	+++	+++	0	0	±	±	0
Anti-horse kidney	0	0	±	±	0	0	++±	++±	+±
Anti-cat blood extract	0	0	±	+	tr	0	++±	++±	+±
Anti-dog blood extract	0	0	±	tr	0	0	++	+±	±

Bloods Nos. 4, 7, 8, 10, and 11 are the same as those in Table 1.

[6] Landsteiner, K., *Wien. Klin. Wschr.*, 1901, 1132.

[7] Landsteiner, K., and Levine, Ph., *J. Exp. Med.*, 1928, **47**, 757.

one or the other of the 2 sorts of sera and such as gave negative or weak reactions with both.

6429

Study of Endocrine Factors Influencing Mammary Development and Secretion in the Mouse.

JAMES T. BRADBURY. (Introduced by H. B. Lewis.)

From the Department of Zoology, University of Michigan.

At sexual maturity the mammary gland of the female mouse consists of the primary duct (galactophore) system. Theelin injections or ovarian implants induce a similar galactophore development in both castrate males and females. No response of the mature female gland is noted after theelin treatment. This is in agreement with the findings of Turner *et al.*,[1] that theelin, theelol or crude estrogenic extracts will not influence the mammae beyond the development of the galactophores. Thus the follicular hormone seems responsible for the establishment of the primary duct system.

It is more or less generally believed that the corpus luteum governs the advanced development of the mammary gland. A satisfactory luteal extract was not obtained. However, luteinization of the ovaries by extracts of the anterior lobe pituitary* or of pregnancy urine† induces development of the secondary ducts (interlobular canals) and later of the alveolar anlagen. In the intact animal this development is much more rapid after pituitary administration than after Antuitrin S. After ovariectomy, neither of these substances will bring about growth of the interlobular canals. Total hysterectomy also inhibits their development even though functional corpora lutea are present in the ovaries. If hysterectomy is done before the appearance of any interlobular canals, they do not appear even after extended periods of luteinizing treatment. Interlobular canals only partially developed at the time of hysterectomy are inhibited from any further growth. Thus it appears that the formation of these secondary ducts is dependent upon the uterus, the activity of which is in turn governed by the corpus luteum.

[1] Turner, C. W., Frank, A. H., Gardiner, W. U., Schultze, A. B., and Gomez, E. T., *Anat. Rec.*, 1932, **53**, 227.

* Pituitary extract whole gland (sheep).

† Antuitrin S.

In the normally developing mammae the alveolar anlagen appear after the interlobular canal system is well established, forming as dense nodular masses which balloon out into grape-cluster lobules when they pass into the secretory phase. Experimentally, pituitary extract induces formation of alveolar tissue in an hysterectomized or ovariectomized animal providing there is some interlobular tissue present at the time of the operation. Antuitrin S is ineffective in the absence of either the ovaries or the uterus.

Hysterectomy, ovariectomy, or both combined, allow the alveoli to go directly into the secretory state as soon as they are formed under the influence of pituitary extract. Interruption of the ovary-uterus complex seems to favor the appearance of milk. In the normal animal it is quite likely that the alveoli form under a pituitary influence but are kept in an inactive state by the presence of some uterine substance which is lost at parturition. Hysterectomy as early as the eleventh day of gestation in the mouse is followed by active milk formation within 48 hours. Frankl[2] inhibited milk secretion at term by placental grafts. It is evident that after the mammae are built up, the lactation stimulus is more effective in the absence of a uterine factor.

In the mouse the mammae regress very markedly after the lactation period. The interlobular canals and the alveoli become greatly atrophied but not completely resorbed. In case of a subsequent pregnancy they regenerate during the same periods that they form *de novo* in the primiparous animal. This progressive regeneration may be interpreted as additional evidence that the components of the mammary duct system are formed under different influences.

The more rapid response to pituitary treatment than to Antuitrin S indicates that it might have a direct effect on the mammae. This is confirmed by the recent report of Riddle, Bates and Dykshorn,[3] claiming to have isolated a substance (prolactin) from the anterior pituitary which acts directly on the mammae.

The extracts used in this study were generously supplied by Dr. E. P. Bugbee and Dr. O. Kamm through the courtesy of Parke, Davis & Co.

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[2] Frankl, O., *Am. J. Ob. and Gyn.*, 1923, **6**, 399.

[3] Riddle, O., Bates, R. W., and Dykshorn, S. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1211.

6430

Atypical Acidfast Organisms.

MAX PINNER.

From the Laboratories, Desert Sanatorium and Institute of Research, Tucson, Ariz.

A number of acidfast organisms has been observed in this laboratory during the last year. As far as could be ascertained by a search of the literature, they have not been described. They fall into 3 distinctly different groups.

Group I. The organisms in this group are air contaminators. These colonies, on various agars, are round, transparent or creamy white, with a diameter of 0.01-1.0 mm. All strains show constantly smooth colonies, with the exception of one which is constantly rough. On smears they are diphtheroid-like, very pleomorphic rods and cocci. The rods are frequently granulated, beaded, barred or clubbed. The cocci vary in size from barely visible granules to coccoidal or diplococcoidal forms of the size of staphylococci. Frequently large single cocci are seen. With the Ziehl-Neelsen method only a part of each smear retains the carbolfuchsin. The relative proportion of the acidfast elements varies greatly with the different strains and with the age of the cultures. Acidfastness is enhanced by growth on egg media. Growth occurs within 24 hours on the usual agar media, in the usual broth media and on tubercle bacilli media; but the addition of dyes, commonly used for the cultivation of tubercle bacilli (gentian violet, malachite green) inhibits growth. In broth, growth occurs throughout, either by even clouding, or slightly granular. All smooth strains are poor fermenters, forming acid but no gas in dextrose only, or not at all. The rough strain ferments several sugars and liquifies gelatine. All strains are completely avirulent for guinea pigs and rabbits, even in massive and repeated doses.

Group II. This group consists of 6 strains, isolated from human material (sputum or urine) or from known strains of human tubercle bacilli. Their outstanding characteristic is their completely smooth, unpigmented colony formation. The colonies are white and glistening, having the appearance of drops of cream, a consistency of butter or cream cheese. They are easily suspended in water. Varying with the strains, the smears, stained by the Ziehl-Neelsen method, show acidfast rods, indistinguishable from typical tubercle bacilli, or mixed with such rods, tiny acidfast and non-acidfast

granules. They grow more rapidly than true tubercle bacilli, some develop visible colonies in 18 to 24 hours. Their growth is luxurious on the usual tubercle bacillus media, but the most rapidly growing strain prefers dextrose to glycerole. On subcutaneous injection in large dosage into guinea pigs, they cause the formation of local abscesses, which have a tendency to spontaneous healing. In addition, strictly localized lesions may occur in the various lymph nodes, in the liver, spleen, lung, omentum, testes and peritoneum. All these lesions—tubercular in gross appearance—have a marked tendency to spontaneous regression. Histologically they consist of a granulation tissue which simulates closely the early phases of tuberculosis; caseation, as a rule, is absent, but abscess formation occurs, particularly in the local lesion at the site of injection. During the first few weeks following the infection, numerous acidfast rods are present in the foci, and can usually be isolated from them in pure cultures; later on, they disappear, and cultures remain sterile more frequently.

It is believed that these strains are probably smooth human tubercle bacilli. Two of these strains were isolated from known tuberculous material, one was found in association with typical rough tubercle bacilli. The other 4 strains were derived directly from typical, known strains of human tubercle bacilli. One strain has on several occasions, reverted to a rough strain following animal passage.

Group III. This group consists of 11 strains, all of which were isolated from human secretions or excretions. Four were sent to me by Dr. J. F. Norton, Detroit, and one by Dr. H. S. Willis, Northville, Mich. These organisms have smooth, glistening, round colonies which show strong lemon yellow to orange pigment. Their color is similar to that of *B. phlei* or of the bacillus of rat leprosy. The consistency is soft creamy to viscous. The individual organisms are acid- and alcohol-fast rods, somewhat larger than tubercle bacilli; they are frequently beaded and granular. On subcutaneous injection they produce lesions in guinea pigs, which in gross appearance and distribution are not unlike those in Group II. They cause frequently perisplenitis, perihepatitis and intestinal adhesions. The lesions are self-healing. Histologically, the foci consist of a non-specific, non-caseating granulation tissue, composed of strikingly polymorphous cells. At times, the center of the focus is an abscess. Acidfast rods are found in great abundance in the lesions before regression sets in. Following reinfection with the homologous strain—2 to 4 weeks after the primary infection—visceral

lesions are much more abundant than after a single infection. Although the infected animals become skin sensitive to tuberculin and to the homologous strain, reinfection does not produce a local allergic reaction. The local nodule which develops promptly on primary infection, frequently fails to develop at the site of reinfection. The pathogenic action of these strains has been compared with that of known non-pathogenic acidfast bacilli, namely, one pigmented acidfast rod isolated from tap water and *B. phlei*. Both these strains produce a similar yellow pigment, but their colonies are rough and dry. Massive doses of these 2 strains produced abscesses at the site of injection, but never any visceral lesions.

The 11 strains of this group are apparently not identical; they vary in growth intensity, in pigment, in their growth on liquid media and in their pathogenic action. Whether they have any significance in human pathology remains to be studied.

6431

Observations on Various Insulin Mixtures Administered Per Os.

ELMER S. GAIS. (Introduced by L. Gross.)

From the Laboratories of the Mt. Sinai Hospital, New York.

At the time of publication of Stephan's paper[1] on the use of "cholosulin", a desoxycholic acid-insulin mixture, we were engaged in the preparation and study of a similar compound based on identical theoretical considerations. Mixtures of desoxycholic acid and insulin were prepared and administered by stomach tube to fasting rabbits, the experiments controlled by subcutaneous injection. The results in 4 experiments with 4 rabbits each, were inconclusive. The blood sugar curves closely paralleled the controls. The recent reports of Bronkhorst, Freud and Laquer,[2] and Wahncau and Bertram,[3] seem to show some slight effect on carbohydrate metabolism by this compound, but they ascribe it to the bile acid and not the insulin in the mixture. The clinical use of Stephan's preparation by Umber and Rosenberg,[4] and others has not proven successful. Our mixture also had no effect on human diabetes.

[1] Stephan, R., *Munch. Med. Woch.*, 1929, **76**, 1579; *Med. Klin.*, 1930, **26**, 228.

[2] Bronkhorst, A. J., Freud, J., de Jongh, S. W., and Lacquer, E., *Nederl. Tijdschr. v. Geneesk*, 1930, **74**, 2185; abs. *Endokrinol.*, 1931, **8**, 43.

[3] Wahncau, E., and Bertram, F., *Klin. Woch.*, 1931, **10**, 486.

[4] Umber, F., and Rosenberg, M., *Deut. Med. Woch.*, 1930, **56**, 169.

Another line of approach to the problem of a practical and successful method of administering insulin by mouth suggested itself. In order to attempt the prevention of digestion of insulin in the gastro-intestinal tract, a preparation from the juice of ground and pressed *Ascaris lumbricoides* of the pig, yielding a potent, assayable anti-protease fraction, was made by the method of Weinland.[5] This substance, a fluffy, white, sticky powder, was assayed in potency by titrating it against active peptic and pancreatic proteases. In definite quantities it prevented the digestion of egg-white and casein. The action is inhibitory and not permanent, lasting about 24 hours.

When a known concentration of this anti-protease was added to mixtures of commercial insulin plus either peptic or tryptic proteases, the potency of the insulin as tested by subcutaneous injection into 8 fasting rabbits, was unimpaired. Typical hypoglycemic reactions resulted. Controls of the proteases, the anti-protease and digested insulin-protease mixtures, gave no hypoglycemic response. In other words, the anti-protease was shown to be capable of preventing the digestion and destruction of commercial insulin by the proteases *in vitro*.

When, however, the mixture of insulin and anti-protease was fed by stomach tube to fasting rabbits (2 groups of 4 animals) in an attempt to prevent the digestion of the insulin in the gastro-intestinal tract, no hypoglycemic reactions were observed. It cannot be stated whether absorption had taken place, for the recovery of the introduced insulin was not attempted. At least one may state that, although large quantities of anti-protease were used, there was no evidence of insulin absorption and action as judged by the blood sugar curve.

The handling of the Ascaris and its extracts causes sensitization phenomena, such as bronchospasm, rhinorrhea, urticaria, etc. Ransom, Harris and Couch[6] noted that the globulin fraction of the tissue juice does not contain the sensitizing substance. Whether this globulin fraction contains the anti-protease would be of interest in the utilization of the anti-protease in man, for the ingestion of the anti-protease powder by the author caused rather severe asthma and uveal and laryngeal edema in the one diabetic to whom the insulin anti-protease mixture was administered. An assistant developed urticaria at first contact with the powder.

Before further human study can be pursued, a sensitizer free ex-

[5] Weinland, E., *Z. Biol.*, 1902, **25**, 86.

[6] Ransom, B. H., Harris, W. T., and Couch, J. F., *J. Agric. Res.*, 1924, **28**, 577.

tract must be prepared. Rabbits, however, are not sensitive and can be used.

Further study of a sensitizer-free insulin anti-protease mixture seems to be warranted. Other sources of the anti-protease are being investigated. Perhaps, if combined with some substance aiding absorption such as desoxycholic acid or saponin,[7] the insulin polypeptide, protected from hydrolysis, might be absorbed and exert its effect when administered *per os.*

6432

Fermentation of the d- and l- Forms of Arabinose by Bacteria.

STEWART A. KOSER AND FELIX SAUNDERS.

From the Departments of Hygiene and Bacteriology and of Physiological Chemistry, University of Chicago.

Although the commoner sugars have found an every day use in bacteriological technic for the separation and characterization of different types of bacteria, it is only rarely that optical antipodes have been subjected to comparative fermentation tests for the purpose of correlating sugar structure with utilization.[1] In most cases it is difficult or impossible to prepare both the *d*- and *l*- forms of a given sugar. Arabinose is one of the few exceptions to this rule.

The common form of arabinose is *l*-arabinose[2] ($[\alpha]_D = +105°$), which occurs naturally in a combined form in many gums such as arabic and mesquite. It has been used in bacteriological work for a number of years. *d*-arabinose seldom occurs naturally and must be prepared from *d*-glucose by degradation. Recently we obtained* a supply of *d*-arabinose which had been prepared by the oxidation of calcium gluconate with hydrogen peroxide in the presence of ferric acetate. This afforded us an opportunity to study the fermentation of both *d*-arabinose and *l*-arabinose, which are exact opposites in configuration and rotation.

[7] Collens, W. S., and Goldzieher, M. A., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 756.

[1] Kendall and Gross, *J. Infect. Dis.*, 1930, **47**, 249. Lester, *Acta Path. Microbiol. Scandanavica*, 1926, **3**, 696.

[2] The symbols *d*- and *l*- in connection with sugars refer to family relationships and not to the sign of rotation. See Rosanoff, *J. Am. Chem. Soc.*, 1906, **28**, 114.

* We are indebted to Dr. W. C. Austin of the Department of Physiological Chemistry of Loyola University for the supply of *d*-arabinose.

The sugar solutions were sterilized by filtration through Seitz filters and added to nutrient broth to give a 1% solution of sugar in the culture medium. After inoculation the various cultures were held at 37° and observed at frequent intervals for 3 weeks. The results are shown in the accompanying table.

The common *l*-arabinose was fermented rapidly by many bacteria. It is quite striking that the synthetic *d*-arabinose was fermented with difficulty by all of the types which utilized *l*-arabinose readily. An interval of 4 to 7 days, or occasionally longer, was required for the break down of the *d*-arabinose to acid end products. Since all cultures developed readily in the *d*-arabinose broth it seems certain

TABLE I.
Fermentation of the 2 forms of arabinose.

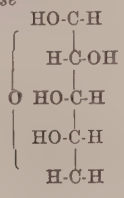
Organisms	No. Strains used	*l-arabinose**	*d-arabinose*
Proteus vulgaris	3	0	+
B. coli	5	+	+ (2)
B. aerogenes	6	+	+ (3 to 5), 1 culture negative
B. friedländeri	4	+	+ (4), 1 culture negative
S. paratyphosum	1	+	+ (5)
S. schottmülleri	2	+	+ (5 to 14)
S. aertrycke	2	+	+ (3 to 8)
S. enteritidis	2	+	+ (3 to 4)
S. cholerae-suis	2	0	+ (6 to 10)
E. typhi	2	0	0
E. dysenteriae, Flexner	2	+	0
E. dysenteriae, Sonne	2	+	+ (8 to 10)
Coryn. diphtheriae	2	0	0
Sarcina lutea	1	0	0
Staph. aureus	1	0	0
Staph. albus	1	0	0
Streptococci, various	6	0	0
Pneumococci, I, II, III	3	0	0
B. subtilis	1	0	0
B. megatherium	1	0	0
Yeasts			
Sacc. cerevisiae	1	0	0
Torula cremoris	1	0	0

+ = prompt fermentation as shown by production of acid, or both acid and gas within 24 hours.

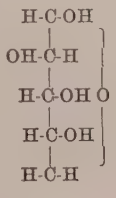
+ ( ) = slow fermentation. The figures in parenthesis give the number of days elapsing before a definite positive test appeared.

0 = no evidence of fermentation.

*Formula for *l-arabinose*



For *d-arabinose*



that the slow fermentation was not due to any restraining effect upon growth of the culture. Fermentation occurred after the peak of the growth curve had been passed.

The behavior of several of the bacteria deserves a special word of comment. The 3 strains of *Proteus vulgaris* all failed to ferment *l*-arabinose but promptly used *d*-arabinose, and seemed to be unique in this respect. This is essentially similar to the finding of Moltke[1] who reported in his monograph on the Proteus group that none of his cultures fermented *l*-arabinose but that all of them fermented *d*-arabinose. It is interesting that such vigorous fermenting types as *Bacterium coli* and *Bact. aerogenes*, both of which are able to break down many varied types of sugars, experienced some difficulty in handling *d*-arabinose. *Salmonella choleræ-suis* is unable to ferment *l*-arabinose and this characteristic has been used as one of the means of separating it from other members of the Salmonella, or paratyphoid, group. It is interesting that this organism produced a delayed fermentation of *d*-arabinose and thus its behavior toward this form of arabinose is similar to that of the other members of the Salmonella group.

A comparison of the 2 forms of arabinose, together with a consideration of the behavior of the organisms toward other pentoses and hexoses, fails to afford an explanation of the results. It is planned to investigate the intermediate metabolism of these sugars in more detail.

6433

Anomalous Lacrimation.

W. F. HAMILTON.* (Introduced by E. B. McKinley.)

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville, Kentucky.

A medical student, A. B. B., has been puzzled because during defecation and sometimes during urination, he has noticed a copious flow of tears. Inquiry has indicated that the experience was at least relatively rare, and he has been unable to find reference to this response in the literature.

[1] Moltke, Contributions to the characterization and systematic classification of *Bact. proteus vulgaris*. Levin and Munksgaard, Copenhagen, 1927.

* Now of George Washington University, Washington, D. C.

He was persuaded to record carefully the intensity of the response in relation to the situation which elicited it. He has made 508 observations during 94 days, the salient facts of which are detailed below.

A "drop" is defined as the amount of fluid necessary to fill the eye and run over onto the cheek. If enough fluid was produced to cause one drop to fall off of the cheek, this was considered 2 drops, or if more than one drop ran off, each was added.

It appears that defecation is a much more effective stimulus (2.2 drops, average of 86 observations) than urination alone (0.01 drops, average of 255 observations) or urination during a desire to defecate (0.4 drops, average of 114 observations), that performing the 2 acts at the same time (2.3 drops, average of 57 observations) does not enhance the response to as great a degree as does defecation with effort to retain urine (3.7 drops, average of 7 observations). The passage of watery feces is much less effective as a stimulus to lacrimation (0.6 drops, average of 5 observations) than is the passage of normal feces. In addition it may be remarked that straining alone has no effect. Warm soap suds enemata and mechanical stretching of the anal sphincter are also without effect.

That the response involves the normal nervous outflow (parasympathetic) is indicated by the fact that the lacrimatory response is eliminated as a result of a moderate dose of atropine.

6434

Treatment of Pin Worm (Enterobius vermicularis) Infestation with Hexylresorcinol.*

H. W. BROWN.† (Introduced by P. D. Lamson.)

From the Department of Pharmacology, Vanderbilt University School of Medicine.

The removal of *Enterobius vermicularis* is especially difficult because its life span of 3-4 weeks is spent in migrating down the small and large intestines. Because of this migration, treatment must be directed against the young worms in the small intestine as well as the gravid females in the large intestine. That the eggs are infective when passed in the anal region by the female worms adds to the difficulty, and the possibility of reinfestation during treatment can-

* The funds for carrying out this work were given by the International Health Division of the Rockefeller Foundation.

† Received for publication July 29, 1932.—Editor.

not be ruled out. Thus in the present study, enemas from one case were negative over a period of 2 weeks and then 7 female worms, all just mature and of the same size, were recovered. From another case negative for 7 weeks, 3 worms were later removed. These worms undoubtedly represented reinfestation during the treatment.

Crystalline hexylresorcinol has been shown by Lamson *et al.*[1] to be very effective against ascaris and hookworm. Further it has been found that a 1-1000 saline solution suspension of this drug kills enterobius in 2 minutes and hence it was tried on a number of cases harboring this worm.

Treatments by Oral Administration and Enemas. Six persons from 3 to 30 years of age were given the combined treatment of crystalline hexylresorcinol in pills by mouth and enemas composed of 1 gm. hexylresorcinol in 1000 cc. of water. The oral dose was 0.1 gm. per year of age up to 10 years, with the maximum dose of 1.0 gm. It was given 8 hours after a light breakfast‡ and no food was then taken for 4 hours after treatment, as it has been shown (Lamson[1]) that this drug combines with proteins and greatly decreases its activity. A preliminary cleansing soap suds enema preceded that of the hexylresorcinol suspension; the latter was given high and retained 5 minutes. The number of treatments varied from 3 to 13 and were at first given twice weekly and the last 3 were given at intervals of 1, 2, and 4 weeks. The patients were advised to boil their bed sheets twice weekly to kill any eggs or worms deposited upon them. The results from the enemas were collected separately, washed through a 40 mesh screen and the worms counted. Since enterobius does not usually pass eggs until it migrates to the anal region, stool examinations for eggs in determining the presence of this worm are not satisfactory. Because of this, one has to rely on the patient examining each stool for the adult worms and on the results of enemas to ascertain whether or not worms are still present.

Table I gives the results of the treatments. Ninety percent of the worms recovered were removed by the first treatment and 99% by three treatments. The greatest number of worms recovered from one person was 1770. Two cases became negative after a single treatment. Five of the cases remained negative for a period of 7

[1] Lamson, P. D., Brown, H. W., Robbins, B. H., and Ward, C. B., *Am. J. Hyg.*, 1931, **13**, 803.

‡ The clinic patients were not available until afternoon. It would probably be more satisfactory to do the treatment at breakfast time, omitting this meal entirely.

TABLE I. *Enterobius (Oxyuris) vermicularis*

Treatment with soap suds enema followed by enema of 1 gm. hexylresorcinol to 1000 cc. of water. Pills of hexylresorcinol by mouth. Number of worms recovered in enemas.

Age	Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	Total	Soap suds enemas	Hexylresorcinol enemas
	Day of treatment	0	2	7	9	14	16	21	23	28	35	42	56	86			
30		19	0 HR*		0										19	8	11
29		9 HR	5 HR	30 HR	1 HR	1	1 HR	2	7	2	0	0	0	0	58	13	45
27		5	1 HR	7 HR	1 HR	1 HR	1			1		0	0	0	17	8	9
9		973	9 HR	2 HR	0 HR	6 HR	1	0 HR	0	0	1	0	0	3	995	8	987
5		1602	40 HR	121 HR	0 HR	0 HR	0	7 HR	0	0	0	0	0	0	1770	280	1490
3		6	0 HR	0 HR	0 HR			0			0	0	0	0	6	1	5
	Worms recovered	2614	55	160	2	8	3	9	7	3	1	0	0	3		318	2547
	% of all worms recovered	90%		99%													

TABLE II.

Treatment with soap suds enema followed by enema of 1 gm. hexylresorcinol to 1000 cc. of water.

7	No pills	87	3	21	3	4	1	3	0	1	8	3	1	1	136	16	120
6	" "				16	1	1	0	1	1	0	0	0	0	20	7	13
4	" "				1	3	0	2	0	0	0	0	0	0	6	3	3

*HR indicates oral administration of hexylresorcinol on this day.

weeks. The one case in which 3 worms were removed after being free of worms for 7 weeks had evidently become reinfested. In these 6 cases, the total worms recovered in the soap suds enemas was 318 and they were all alive, while the 2547 recovered in the hexylresorcinol enemas were all dead. These figures represent only the worms recovered in the enemas and, of course, do not include worms killed but not expelled in the enemas as well as those killed by the oral administration of the drug and expelled sometime later.

Treatment by Enemas. Three cases were treated by enemas only, omitting the pills by mouth. Table II shows that 2 of the cases became free of worms after 3 and 5 treatments respectively, while the third case was still positive after a series of 13 enemas over a period of 3 months. Since the life span of the worm in man, at the most, is probably not over 3 to 5 weeks, it is quite obvious that in this case we were dealing with a series of reinfestations.

Treatment by Oral Administration. Six adults harboring enterobius were each given 1.0 gm. of hexylresorcinol pills. The drug was given early in the morning and breakfast omitted and no food was taken until 6 hours later. Three weeks after treatment, stool examinations were made and all 6 contained enterobius or its eggs.

Conclusion. 1. Hexylresorcinol is very active on *Enterobius* vermicularis, a 1-1000 suspension killing them in 2 minutes. 2. Five out of 6 people treated with hexylresorcinol pills by mouth and enemas became free of their enterobius. Two out of 3 people treated by enema only became free of enterobius. Although this is not sufficient data to judge which method is the best, the life history of the worm indicates need of both oral and enema treatment. 3. Suggested outline of treatment. Treat twice a week in the morning as follows: (a) Omit breakfast; no food until noon. (b) Hexylresorcinol pills orally, 0.1 gm. per year of age up to 10 years of age, maximum dose after this age 1.0 gm. Drink plenty of water. (c) Soapsuds enema and after its evacuation an enema of 1 part crystalline hexylresorcinol in 1000 cc. of water; this enema to be given high and retained 5 minutes. (d) Bed sheets to be boiled at least twice weekly to destroy eggs or worms passed on them.

6435

On the Motion of Growth. I. Introduction to the Energetics of Growth and Metabolism.

NORMAN C. WETZEL.

From the Babies and Childrens Hospital, Cleveland, and the Department of Pediatrics, School of Medicine, Western Reserve University.

It is proposed in this and in a few succeeding papers briefly to present the major results of an extensive investigation into the general nature and mechanism of growth, a problem which has now occupied our attention for the past eight years. It is clearly impossible, accordingly, to do more here than to sketch in the barest fashion such features as are of primary interest to those working in this field. A detailed description whereby all steps in the argument are properly and fully justified has already been prepared for early publication elsewhere.

Previous workers as described by Scammon[1] have contributed

[1] Scammon, R. E., Report of National Research Council Committee on Child Development, Washington, 1929, Part I, pg. 1.

greatly in diverse ways to our knowledge of the outward, graphical characteristics of growth in various biological forms, but it needs to be pointed out that no analyses have been brought to the point where the intrinsic properties or perhaps better, the special mechanisms of growth as such are thereby satisfactorily clarified, and further, that there is nowhere a hint as to the real connections of this fundamental process with other biological processes as, for example, that of metabolism. In the present instance we have founded our entire procedure upon the general, and indeed, almost obvious concept that all growth must be viewed as a process of biological energy exchange. Primary stress is therefore to be laid upon the importance of dealing with this entire subject from the standpoint of what may be called the energetics of growth. The *dynamical* association of growth with metabolism comes thus as a natural result of this principal postulate.

A General Concept of the Energetics of Growth.

With due respect to various possibilities in the task, we begin with a broad definition of growth and take this in the biological as well as in the dynamic sense to refer primarily to a *change* in cell number. Such a change, however, need not always be an increase in cell number, although under ordinary circumstances, obviously, the characteristic change will be that associated with, and due to, cellular reduplication. It is next assumed that any biological entity undergoing growth is in suitable contact with an appropriate source of energy; and that the chief products, daughter cells, may find agreeable accommodations in their immediate surroundings. Thus, source, cells, and environment when properly adapted with respect to subsidiary physical conditions,* will constitute for simplicity a system wherein growth proceeds in accordance with these several adjustments. It is further assumed that the energy so withdrawn from the source becomes transformed in part into daughter cells, this fraction comprising the *"Internal Work of Growth,"* and in part into other fractions which together compose the *"External Work"* of this process. Thus, by the conservation law we have schematically,

$$\left[\begin{matrix}\text{Energy of the}\\ \text{Source}\end{matrix}\right] \rightarrow \left[\begin{matrix}\text{Internal Work}\\ \text{of Growth}\\ \text{(Daughter Cells)}\end{matrix}\right] + \left[\begin{matrix}\text{External Work}\\ \text{of Growth}\\ \text{(Loss by Dissipation,}\\ \text{Energy of Storage,}\\ \text{Synthesis, etc.)}\end{matrix}\right] + \left[\begin{matrix}\text{Work of}\\ \text{Maintenance}\end{matrix}\right]$$

* Temperature, $[\overset{+}{\text{H}}]$, etc., etc.

where provision has also been made for cell nutrition or maintenance. The proper mathematical representation of the foregoing concept ought accordingly to include all cases of growth—and, if appropriate solutions can be obtained, might also to lead to a clearer understanding of processes hitherto considered unrelated to the main event itself.

Our own studies have shown that the general relationship given above can be expressed, when the quantities have been properly chosen, by the equation,

$$\underbrace{S - \int V_c(t)\,dt}_{\text{Internal Work}} = \underbrace{\int \left(E_s(t)\,dt + r\left(\frac{dq}{dt}\right)^2 dt\right) + \frac{q^2}{2\sigma} + \frac{\lambda}{2}\left(\frac{dq}{dt}\right)^2}_{\text{External Work}} + \underbrace{Mt + C_0}_{\text{Maintenance}} \quad (1)$$

wherein the various factors are defined as follows:

- q, the dependent variable, and referred to as the "charge of growth" later to be shown equal to $k\int \frac{1}{z}\frac{dz}{dt}dt$, z being a function of cell number, and k a constant of proportionality herein taken as unity;
- S, Energy at the Source;
- $V_c(t)$, Energy appearing in the cells themselves;
- r, a constant accounting for the true irreversibility of any finite growth process, and termed the Resistance to Growth;
- σ, a constant to be known as the Permittance of Growth;
- λ, a constant pertaining to the momentum of growth;
- E_s, a constant representing the Energy of cellular synthesis per unit charge;
- M, the unit rate of maintaining cells;
- C_0, the arbitrary constant of integration.

Due to the ever increasing complexity of the subject, spatial relations in any of the three dimensions are neglected as unimportant in a first approach to the problem. With this restriction the foregoing relation represents the full expression of the fundamental agencies which together accomplish the work of growth. We refer to it as the equation of energy, and each of its terms will be separately justified and developed in another series of forthcoming papers.

Results—Equation (1) leads directly, when the functions S and $V_c(t)$, $E_s(t)$ are known[1] to a solution of an integral in t for the change in weight of the human being from the 100th day of gestation to full adult life, a longer period than heretofore embraced in

[1] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, 30, 227.

any single analytical or "rational" expression for weight. It leads next by way of the numerical evaluation of ρ to a description of the course of human basal metabolism throughout the same period of life,[3] in terms either of unit or total heat production. Some insight is thus gained into the curious succession of critical points shown in growth and metabolism respectively. This expression for the normal alterations in human metabolism as age proceeds through the period of growth will also serve to define the course of heat production during starvation.[4] In addition, equation (1) may be so arranged to account, at least dynamically, for the nature of the change in weight incident to prolonged fasting, the human case being considered briefly in a succeeding paper.[5] Lastly, equation (1) has been applied to numerous other examples of growth including that displayed by tissue cultures, yeast cells, plants and animals, and in all of these it has succeeded in agreeing with experimental observations. A special example, where, fortunately, simultaneous data on heat production are also available, is considered for the case of bacteria in the final paper of this series.[6]

6436

On the Motion of Growth. II. Human Growth in Weight from Early Fetal to Adult Life.

NORMAN C. WETZEL.

From the Babies and Childrens Hospital, Cleveland, and the Department of Pediatrics, School of Medicine, Western Reserve University.

The equation of energy previously set forth[1] has been applied to the case of human growth in weight but the steps necessary for this can only be briefly outlined here. We need first to know the nature and relations of the functions $S, (V_c, E_c)(t)$, which as already explained, pertain to energy at the source, to that in the form of cells, and to the energy of synthesis respectively. It can be shown that these several factors are connected in the case of "average" human weight by the relation,

[3] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 233.
[4] Wetzel, N. C., to be published.
[5] Wetzel, N. C., to be published.
[6] Wetzel, N. C., to be published.
[1] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 224.

$$S-\int [V_c+E_c](t)dq = \int [E-E_o\epsilon^{-\beta t}\sinh(\theta t-\zeta)]dq+A't \qquad (2)^*$$

E, E_o, β and θ being constants and $\zeta = \tanh^{-1}\frac{\theta}{\beta}$. Certain other examples of human growth which depart from the "average" healthy trend, especially between 6-16 years of life have actually proven to be represented instead by an analogous circular function on the right of (2) though this exceptional case will not be treated in detail now. But it is of considerable interest that, whereas neither the hyperbolic nor the circular terms can be omitted in the human case, they vanish for all other examples of plant and animal growth we have so far studied.

The identity (2) is thus seen to symbolize merely the *balance* of energy after that which provides for the cells themselves, and that which is to do the work of synthesis has been deducted from the source. This balance must now suffice to perform three remaining fractions which, together with the energy of synthesis (already discounted), constitute the external work of growth.

Substituting, then, into the equation of energy (1), differentiating once with respect to t, and transposing terms we get an equation each term of which by these procedures figuratively denotes, per unit of weight, a component in the "forces" of growth:

$$\lambda\frac{d^2q}{dt^2}+\rho\frac{dq}{dt}+\frac{q}{\kappa} = E-E_o\epsilon^{-\beta t}\sinh(\theta t-\zeta) \qquad (3)$$

the change in dimension just described being noteworthy. In virtue of (2) it may therefore be seen that equation (3) actually represents the balance of "forces" (and fundamentally, of energy also) remaining to provide on the left for the forces now recognized respectively, as that concerned with the creation and maintenance of the momentum of growth, that required to "overcome" the resistance of growth, and finally that involved in the storage of growth in an environment of permittance, κ.

The foregoing equation has three main integral solutions, accounting theoretically for three outwardly different, though fundamentally identical types of growth, defined in general by the relation,

$$\psi = \left[\left(\frac{\rho}{\lambda}\right)^2-\frac{4}{\lambda\kappa}\right] \gtreqless 0 \qquad (4)$$

where, as a rule, $\psi<0$ in the case of bacterial growth, but where

* To facilitate reference the equations in this series of papers are numbered serially.

$\psi > 0$ in human growth. Hence, in the latter, the auxiliary roots are real and negative, and the complete solution of (3) including both complimentary function and the particular integral, becomes by the usual methods, in most compact form,

$$q = E\kappa + De^{-\beta t}\sinh(\theta t + \phi) + C_1 e^{-a_1 t} + C_2 e^{-a_2 t} \qquad (5)$$

the arbitrary C's, the a's and D being developed in terms of λ, ρ, and κ, in expressions too bulky to be given here, and ζ being taken up into ϕ.

Now the constants λ, ρ, and κ, cannot be themselves obtained from any analysis of growth curves alone. Quite independent relationships are necessary for this,† but the computation of values for the human curve in weight can nevertheless be completed as soon as the "combined" constants ($E\kappa$), D, C_1, C_2, a_1, a_2, and the single constants θ and ϕ are known, for these may be obtained exclusively from data on growth by a generally applicable method involving the use of least squares subsequently to be described,[2] and are, for our Series XIX:

$E\kappa =$ 4.397966	$a_1 =$ 3.737101
$D =$ 9.518210	$a_2 =$ 0.143894
$C_1 =$ —11.030331	$\theta =$ 0.19
$C_2 =$ — 5.578521	$\beta =$ 0.51
	$\phi =$ 0.217884

when q is expressed in Napierian logarithms. The final figures of all but θ and β are rounded.

Of interest is the fact that every step in the calculations, both in deriving the original values just set out, as well as in computing final results for q or z at any time t must be carried through with no less than six, preferably eight to ten significant figures to insure accuracy. The final results for weight, of course, are expressed only to four significant figures, and are set up for various ages in Table I. They are also displayed graphically in Figures (1) and (2) for the ante- and post-natal phases respectively. The former, due to the tremendous change in weight, is drawn semilogarithmically, and shows clearly that relative deviations even in the earliest stages where these tend to be greatest are in reality negligibly small. The data are those of Streeter.[3] For the post-natal phase the ordinary grid is used to avoid confusion in view of the fact that all such

† See following paper (III).

[2] Wetzel, N. C., to be published.

[3] Streeter, G. L., Carnegie Institution of Washington Publication No. 274, Contributions to Embryology, 1920, **11**, 143.

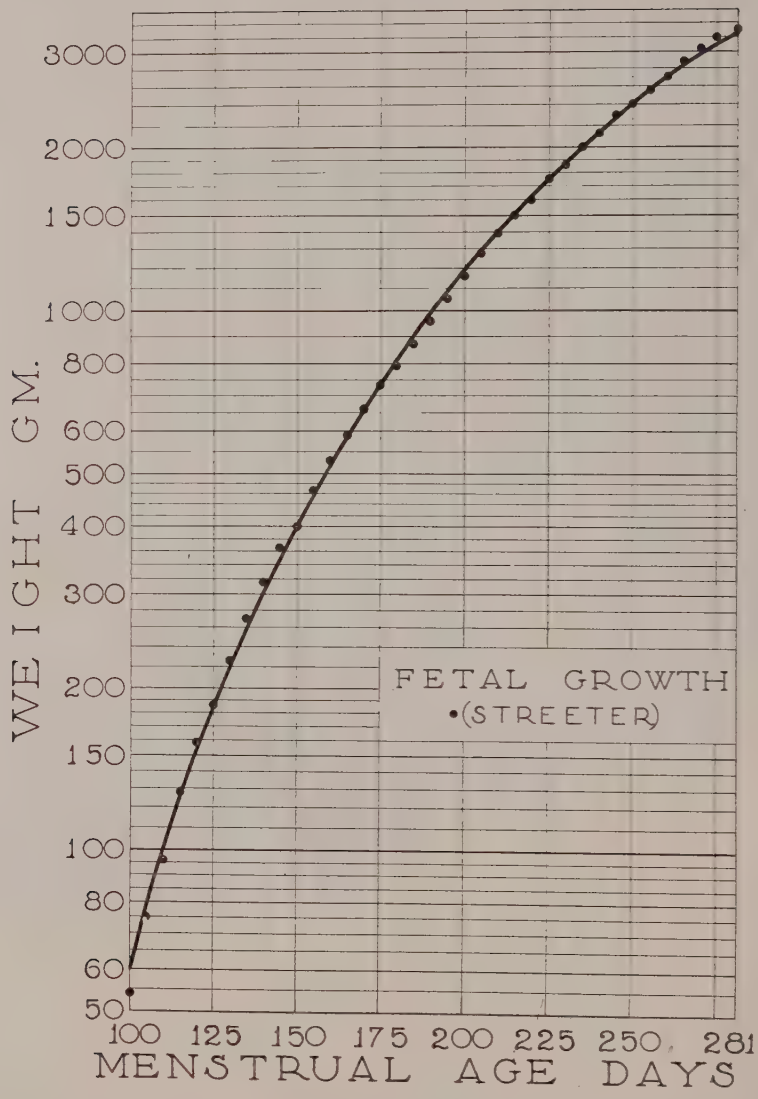


FIG. 1.

The smooth curve displays the prenatal course of growth in weight from the 100th day of gestation (menstrual age) to birth and passes directly through the values computed from equation (5) for this period. The adjustment to the data of Streeter is entirely satisfactory.

TABLE I.
Giving the Numerical Values of Average Human Weight and Rate of Growth as Computed by Means of Equation (5) and its Corresponding Derivatives.

	Age	Weight (z)	Rate of Growth	
			dq/dt	dz/dt
		Kg.	*Kg./Kg./year*	*Kg./year*
Menstrual Age	100 days	0.069	16.0539	1.108
	125 "	0.185	12.6366	2.338
	150 "	0.400	9.9786	3.992
	175 "	0.736	7.9091	5.821
	200 "	1.194	6.2960	7.517
	225 "	1.757	5.0367	8.850
	250 "	2.396	4.0520	9.709
	281 " (Birth)	3.251	3.1208	10.146
Legal Age	6 mos.	7.565	0.8667	6.559
	1 yr.	10.062	0.3788	3.813
	2 yrs.	12.695	0.1569	1.993
	3 "	14.192	0.0913	1.296
	4 "	15.486	0.0876	1.356
	6 "	18.852	0.1095	2.064
	8 "	23.759	0.1186	2.818
	10 "	29.967	0.1115	3.342
	12 "	36.917	0.0963	3.555
	14 "	44.000	0.0792	3.485
	16 "	50.712	0.0630	3.195
	18 "	56.709	0.0492	2.790
	20 "	61.850	0.0380	2.350
	22 "	66.030	0.0292	1.928
	24 "	69.510	0.0221	1.536
	32 "	77.316	0.0072	0.557

curves have become well known in this form. The field through which the theoretical curve for healthy weights passes denotes the limits of what can actually be accepted as "normal average" in the collected and well-known observations of Camerer, Czerny-Keller, Pirquet, Woodbury, Baldwin, Boas, and others.‡

The agreement, for the purpose in view, namely, a reproduction of the trend of healthy human growth throughout the entire life cycle is wholly satisfactory, notwithstanding the rather remarkable span over which equations (1-5) hold true. They may, accordingly, be confidently trusted even in this instance, which, so far as we are aware, undoubtedly represents the most complicated example of growth. They are, finally, of sufficient generality to be applied, if desired, in similar ways to the case of "individual" growth curves.

‡ The recent excellent data of Gray and Ayres[4] follow the upper limit of this field faithfully.

[4] Gray, H., and Ayres, J. G., Growth in Private School Children, Chicago, University of Chicago Press, 1931.

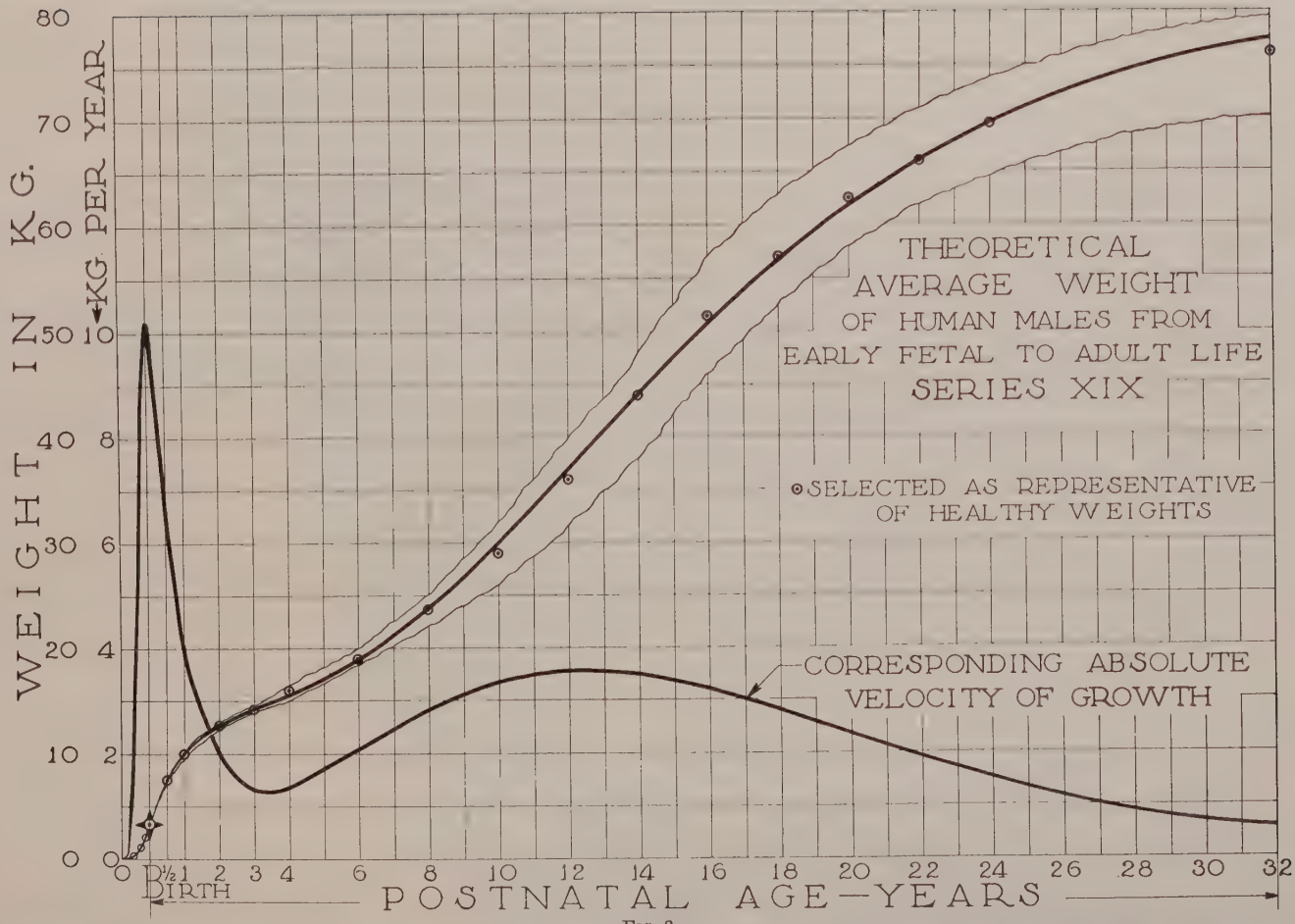


FIG. 2.

The smooth curve is drawn through the values computed directly from equation (5). It redescribes satisfactorily the "ideal" course of growth indicated by the points chosen as representative of healthy weights from early prenatal throughout late postnatal life. The former phase is displayed greatly magnified in Fig. 1. The surrounding white field would contain 98% of all data available for healthy subjects,

6437

On the Motion of Growth. III. The Determination of ρ and the Energetics of Human Basal Metabolism.

NORMAN C. WETZEL.

From the Babies and Childrens Hospital, Cleveland, and the Department of Pediatrics, School of Medicine, Western Reserve University.

We have already pointed out[1] that none of the coefficients, λ, ρ or κ appearing in the original equation of energy (1) and in all subsequently derived relations can be separately determined from an analysis of data on growth alone. All that can be done is to calculate the ratio $\left(\frac{\rho}{\lambda}\right)$, and the product $(\lambda\kappa)$. But, if, as also noted, some independent relationship could be found, their computation would in general be a simple matter. Admittedly, the ultimate goal here must be a determination, if possible, not so much of the numerical values of λ, ρ and κ as of their fundamental physical and physiological attributes and counterparts; but from what has already been given,[1] even a knowledge merely of the values of any one of these coefficients would greatly aid in a fuller understanding of the basic mechanism at work. Many observations will, accordingly, need to be repeated with such an end in view.

Thus, in seeking for some independent relation by which any one of these coefficients could be numerically determined, we were attracted almost at once, in view of the purely dissipative character of the energy accounted for in the term $\rho \int \left(\frac{dq}{dt}\right)^2 dt$ to an examination of heat production during life, under conditions necessarily recognized as "basal". Further inquiry showed, moreover, that the term in ρ should not of itself account for the entire heat output, for the energy involved in synthesis as well as that concerned with nutrition and maintenance ought theoretically also to find an ultimate outlet in heat. The energy represented in the remaining terms of (1) could not, clearly, collaborate in this way, being, as it were, "bound" in doing work of another kind. Consequently, if U be taken as the rate of heat production per unit of weight, we have, for the human case, by selecting the terms just described,

$$\rho \left(\frac{dq}{dt}\right)^2 + \left[E_c + E_2 \epsilon^{-\beta t} \sinh(\theta t - \xi)\right] \frac{dq}{dt} + A' = U \qquad (6)$$

if $E_0 = (E_1 + E_2)$. U thus accounts for a portion of the external

[1] Wetzel, N. C., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 227.

work of growth, namely that of dissipation, without which no finite natural process may be accomplished; that involved in the process of cellular synthesis, and finally that required for continued maintenance. Now, while it is impossible to enter here into a complete analysis of this equation, we ought, nevertheless, to point out that U is henceforth unrestricted as to the sign of $\left(\frac{dq}{dt}\right)$, the relative rate of change in mass (weight), and that accordingly, the first fraction on the left is *irreversible heat*, whereas that concerned with synthesis (second term) is necessarily *reversible* heat, depending, however, not only upon the sign of $\left(\frac{dq}{dt}\right)$ but also upon the relative numerical values of E_c and E_2 when $\theta t < \zeta$.

Since all the factors except ρ, E_c and E_2 can be obtained either from the foregoing results in respect to growth, or, in the case of A' and U, from suitable experimental data, it is clear that the numerical value of ρ, primarily, as well as of E_c and E_2 may be computed to as close a degree of approximation as the data allow. Thus, choosing A' and U in the common units of calories per Kg. per day, we have computed by least squares the values given in the first column of Table I from the original data on basal metabolism reported by Benedict and his associates.[2] Due, however, to the

TABLE I.
The Numerical Value of ρ, E_c, and E_2.

	When $A' = 25.3425$ Cal./Kg./Day	When $A' = 38665 \times 10^3$ Joules/Kg./Year
ρ	53.840886	82,145.04
E_c	54.521815	83,183.93
E_2	2,093.144316	3,193,510.28

fact that $\left(\frac{dq}{dt}\right)$ is more conveniently calculated in terms of Kg./Kg. of body weight per year, we have also set up in the second column the corresponding values in terms of Joules per Kg. per year.

As a crucial test of these procedures it is merely necessary to resubstitute the foregoing values for ρ, E_c and E_2, as well as those for the other terms into (6), thereby obtaining the results throughout the entire period of growth given in the third column of Table II,

[2] Benedict, F. G., and Talbot, F. B., Metabolism and Growth from Birth to Puberty, Carnegie Institution Publication No. 302, Washington, 1921. Harris, J. A., and Benedict, F. G., A Biometric Study of Basal Metabolism in Man, Carnegie Institution Publication No. 279, Washington, 1919. Benedict, F. G., *Am. J. Physiol.*, 1928, **85**, 607.

TABLE II.
The Theoretical Values for Human Basal Metabolism from Birth through Adult Life Computed as Described in the Text.

Age	*(Uz)*	*(U)*
yrs.	*Cal./Day*	*Cal./Kg./Day*
Birth	155.13	47.72
6 mos.	446.97	59.08
1 yr.	664.09	66.00
2 yrs.	680.89	53.63
3 "	607.41	42.80
4 "	633.93	40.94
6 "	760.63	40.35
8 "	897.39	37.77
10 "	1041.81	34.76
12 "	1193.49	32.33
14 "	1343.74	30.54
16 "	1481.77	29.22
18 "	1602.00	28.25
20 "	1702.73	27.53
22 "	1782.54	27.00
24 "	1847.57	26.56
32 "	1989.99	25.74

for "unit heat production"—or, when the latter are multiplied by the corresponding values of the weight, z, from (5)—for what may here be called "total" metabolism per day. The respective curves are shown in Figure 1, their trend representing as closely as can be determined the real course through the observational points. Noteworthy here in the case of "unit" metabolism (lower curve) is the comparatively low value at birth, the rise to a maximum just beyond one year of age and the shoulder-like descent during the period of pre-school and school life. This curve for U should especially be compared with that for $\left(\frac{dz}{dt}\right)$ the absolute rate of gain in Figure 1 of the preceding paper.[1] Taken together these two curves demonstrate a remarkable succession of events in the energetics of growth which cannot, however, be thoroughly discussed here.* Likewise of interest in the curve for total metabolism is the curious "double inflexion" between 1 and 3 years of age, yet here—during this critical phase—the trend is unmistakably in the immediate neighborhood of all observational points. This is not the place for a discussion of the possible physiological significance

* Riddle, Nussmann and Benedict[3] have just recently commented upon a similar group of phenomena in respect to heat production per unit of surface. The events described above and displayed in Figure 1 for both unit as well as for total metabolism per day may now be traced directly to the dynamical relationship between growth and heat production as defined by equations (1-6).

[3] Riddle, O., Nussmann, Theodora, and Benedict, F. G., *Am. J. Physiol.*, 1932, **101**, 251.

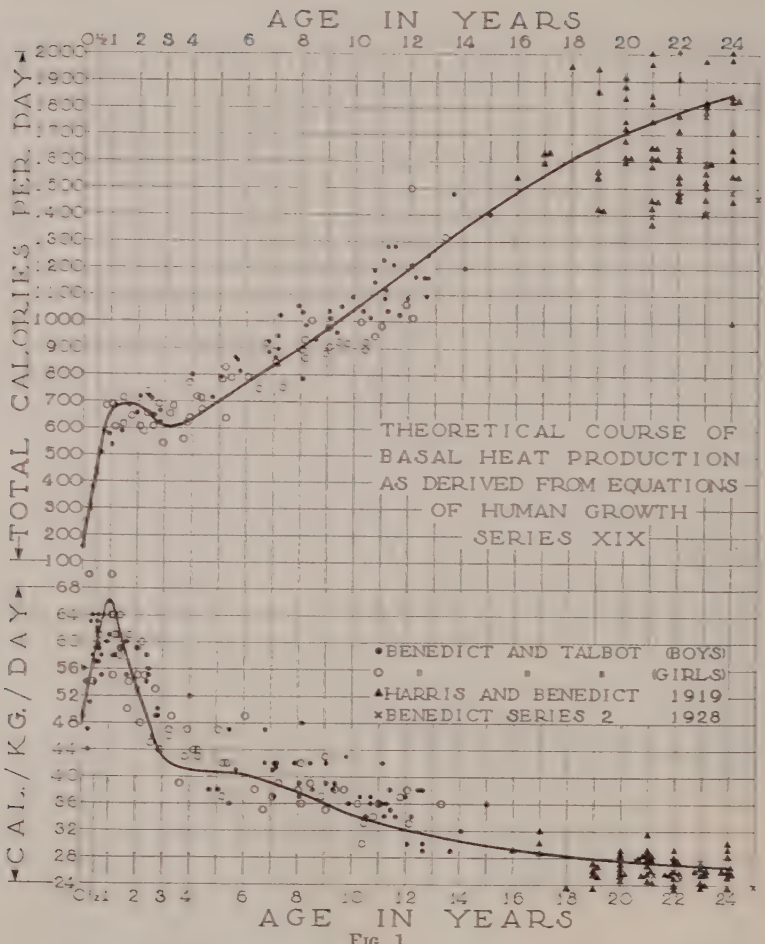


FIG. 1.

The smooth curves display the theoretical trend of heat production from birth to 24 years corresponding to growth (Series XIX).[1] The lower is the curve of equation (6) and represents U; the upper, the curve of (Uz).

of these various features—but it must be stated here that much of the confidence we have placed in these results is to be traced to the fact that we had at our disposal such trustworthy data as those established by Benedict and his coworkers.

6438

Concerning the Naturally Acquired Resistance of the Livers of Certain Senile Dogs to Alcohol and to Chloroform.*

WM. DE B. MACNIDER.

From the Laboratory of Pharmacology, University of North Carolina.

In the preceding report[1] the observation was made that when dogs recovered from an intoxication by uranium nitrate with the development in the liver of a morphologically altered flattened type of epithelium, the liver acquired a variable amount of protection to a secondary intoxication from this substance and also a resistance to the toxic action of chloroform when used as an anesthetic. Of the 161 dogs referred to in this report, 21 may be classified as senile, varying in age from 9 years and 2 months to 14 years and 4 months. Liver tissue removed from 15 of these 21 animals for purposes of control before any type of intoxication had been commenced, other than a light ether anesthesia, has shown a liver injury of unknown cause characterized by a change in the morphology of the liver cells from the normal polyhedral type to an atypical, flattened type with proportionately large, deeply staining nuclei. The capillary spaces between the cords of flattened liver cells are of such a diameter as to resemble venous sinuses. There has been no connective tissue overgrowth. The 15 dogs with this type of altered liver epithelium have shown when contrasted with senile animals with a normal liver epithelium a higher initial plasma concentration of phenoltetrachlorphthalein and a retention of the dye in the plasma for a longer period than animals with a normal epithelium.

Eight of the senile animals with the atypical type of flattened epithelium have been intoxicated by the use of 20 cc. of a 40% solution of ethyl alcohol per kilo for periods varying from 8 to 12 hours. The remaining 7 animals after a 2-day period of starvation have been anesthetized by chloroform for 2 hours. The result of such intoxications has been controlled by 10 normal dogs subjected to the same experimental procedure. The normal control animals intoxicated with alcohol have developed an edema of the liver cells which commences in the periphery and involves the outer one-third to one-half of the lobules. The use of chloroform in such normal control

* This investigation was made possible by the Edward N. Gibbs Prize Fund of the New York Academy of Medicine.

[1] MacNider, Wm. deB., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 78.

animals after a period of starvation has induced a central necrosis of the liver lobules which has varied in extent. The initial concentration and percentage retention of phenoltetrachlorphthalein has been extremely variable, apparently depending upon the extent of the liver injury.

The 8 dogs which were demonstrated to have acquired a morphologically altered type of liver epithelium and later intoxicated by alcohol, and the 7 animals with a similar type of liver cell anesthetized with chloroform, showed both anatomically and functionally a resistance to these poisons. All of the animals which received alcohol as an intoxicant failed to show the characteristic injury commencing at the periphery of the lobules and extending toward the central vein. Five of the dogs anesthetized with chloroform failed to develop a central necrosis of the liver lobules. The 2 remaining animals showed an early injury to this part of the lobules.

The functional expression of this lack of injury to the morphologically altered liver epithelium by ethyl alcohol and chloroform has consisted in a lower initial concentration of phenoltetrachlorphthalein in the plasma and a more rapid disappearance of the dye from the plasma than was the case with the control animals in which the liver epithelium was susceptible to injury from ethyl alcohol and chloroform.

The number of senile dogs with a naturally acquired, morphologically altered type of liver epithelium is insufficient to permit final deductions concerning the resistance of such epithelium to intoxication by ethyl alcohol and chloroform.

6439

Acquired Resistance of Altered Type of Liver Epithelium to Uranium Nitrate and to Chloroform.*

WM. DE B. MACNIDER.

From the Laboratory of Pharmacology, University of North Carolina.

During the past nine years, studies in this laboratory were concerned with the type of liver injury induced in dogs by uranium nitrate and other chemical substances, with the mechanism of repair to the liver lobules and the influence of secondary intoxications by

* This investigation was made possible by the Edward N. Gibbs Prize Fund of the New York Academy of Medicine.

these substances and by chloroform on the regenerated liver cells. A total of 161 dogs of ages varying from 4 months to 14 years have been used. In 73 dogs phenoltetrachlorphthalein has been employed according to the technique of Rosenthal[1] as an index of liver function. The dye was used in normal dogs before the intoxications, during the initial acute liver injury and at the end of a 4-week period, allowed for liver repair and during the secondary intoxication by uranium or when chloroform was used as the hepatoxic agent following a liver repair from uranium. Before such periods of functional study the animals were lightly anesthetized by ether, an incision made below the right costal margin, and liver tissue removed from one or more areas of the organ by means of a nasal cutting forceps or punch. Such tissue was fixed in corrosive-acetic, and in 10% formaline, and sections stained with Scharlach R. for lipoid material.

The uranium intoxications with the resulting liver injury have been induced by the subcutaneous injection of 2 to 4 mg. of uranium nitrate per kilo. The liver injury from uranium is diffuse, involving the cells of the liver lobules as a whole. In a number of animals this injury was not as marked immediately around the central vein of the lobules as it was in their central and peripheral portions. This variation from the diffuse character of the lobular injury may have some influence on the type of epithelial regeneration in the lobules. The injured cells have shown cloudy swelling, edema, vacuolation and a variable amount of necrosis. The severity of injury is associated with the dosage of uranium and the age of the animal. The injured liver cells, regardless of the extent of the necrosis, have shown a marked accumulation of stainable lipoid material, usually in the form of large droplets. The functional expression of this diffuse liver injury from uranium consisted in an increase in concentration of phenoltetrachlorphthalein in the plasma as compared with the same animal before the use of uranium. There is also a retention of the dye in the plasma for a longer period than with normal animals.

At the end of 4 weeks, allowed for liver repair, the animals which recovered from the initial intoxication by uranium were again studied functionally by phenoltetrachlorphthalein, and were then subjected to a second uranium intoxication. On the basis of the data obtained the animals could be divided into 2 general groups. The first showed a normal percentage retention of dye in the plas-

[1] Rosenthal, S. M., *J. Pharm. and Exp. Therap.*, 1922, **19**, 384.

ma followed by its rapid disappearance. Liver tissue from such animals showed repair which in general restored the liver to its normal histological structure.

In the second group the percentage retention of the dye in the plasma was higher, and the dye was retained for a longer time (23 minutes to over 1 hour), repair in the liver had not resulted in the formation of a normal type of liver cell. The type of cell developing as a process of repair in this second group of animals was of a very flattened type, with large deeply staining nuclei. Such cells were found in narrow cord-like structures which radiated in an irregular course from the central vein to the periphery of the lobule. Not infrequently such liver cords failed to show cell differentiation but existed in the form of syncytium. A similar type of epithelial regeneration has been previously described[2, 3] for the kidney following a uranium nephritis.

When dogs in the first group recovered from the initial uranium intoxication, with the regeneration of liver cells of a normal type and with a normal functional response as shown by phenoltetrachlorphthalein, are then given a second injection of uranium nitrate, there is no evidence of such animals developing any resistance for this poison. The liver cells rapidly degenerate and, associated with this change and dependent upon its extent, there occurs a high percentage concentration of phenoltetrachlorphthalein in the plasma and a delayed disappearance of the dye from the plasma. In the second group of dogs, which failed at the end of 4 weeks to show a normal functional response when the dye was used as a liver function test, and which showed the repair of the liver injury from uranium to have consisted in the formation of atypical, flattened cords of liver cells, have also shown a marked resistance to a second intoxication by uranium. This resistance of the liver cell of altered morphology not only occurs when the second injection of uranium is similar in amount to the first injected, but when the amount injected is increased twofold. Such regenerated cells fail to become edematous and vacuolated, the nuclei stain in a normal manner and only a small amount of stainable lipoid material in the form of granules and small droplets can be microchemically demonstrated within the cells.

Chloroform has been given to animals in the 2 groups after 2 days of starvation according to the method of Whipple and Sperry.[4] A

[2] MacNider, Wm. deB., *J. Exp. Med.*, 1929, **49**, 411.

[3] MacNider, Wm. deB., *Science*, 1931, **73**, 103.

[4] Whipple, G. H., and Sperry, J. A., *Johns Hopkins Hosp. Bull.*, 1909, **20**, 275.

similar though less marked resistance has been shown by the altered type of liver cell to an anesthesia from chloroform lasting for 2 hours.

In such animals that have developed a cytological resistance to uranium and chloroform, the percentage concentration of phenoltetrachlorphthalein in the plasma is not as high as it is in those animals with the formation of a normal type of liver cell which has less resistance to a secondary intoxication by uranium or to the use of chloroform for its hepatoxic effect. The removal of the dye from the plasma is more readily effected by those animals with a resistant type of regenerated epithelium than it is by the animals with a normal type of nonresistant epithelium.

6440

Synthetic Diets for Herbivora.

J. W. WOODWARD AND C. M. MCCAY. (Introduced by L. A. Maynard.)

From the Laboratory of Animal Nutrition, Cornell University, Ithaca.

Synthetic diets for herbivora present two special problems, first, because these species are accustomed to diets containing large amounts of cellulose, and second, they are accustomed to high levels of inorganic elements that tend to keep the urine alkaline. The chief problem in selecting a suitable source of pure cellulose is one of physical composition. We attempted to maintain goats upon synthetic diets in which the cellulose portion was furnished by paper, but never succeeded in maintaining the animals upon such a ration. They usually refused the feed entirely after a period of 2 weeks. On the other hand, we have found both goats and rabbits eat regenerated cellulose as readily as the rats, discussed in our earlier report.[1] In devising suitable inorganic mixtures for herbivora we have adopted the plan of imitating the ash constituents of a diet of natural feedstuffs known to be satisfactory for goats and sheep. Such a mixture, however, is rich in the element, silicon. Although it has long been recognized that skin, hair and wool are relatively rich in silicon, we know little of the nutritional requirements for this element. Furthermore, we know little about the form in which it occurs in plants and almost nothing concerning its metabolism in the animal body.

[1] McCay, C. M., PROC. SOC. EXP. BIOL. AND MED., 1929, **27**, 209.

During the past year we have maintained an adult male goat upon a synthetic diet of the following composition, for a period of 152 days: regenerated cellulose* 30, corn starch 30, casein 15, sucrose 10, yeast 5, salt mixture 7, and lard 3. The salt mixture was made by mixing together the following salts and acids: K_2CO_3, 219.0, Na_2CO_3 1.9, $CaCO_3$ 192, $MgCO_3$ 96.6, Fe citrate 20.0, H_2SO_4 30, H_3PO_4 41.8, Na_2SiO_3 97.0, HCl 28.5, $CuSO_4$ 0.2, KI 0.03, $MnSO_4$ 0.12, NaF 0.37, K alum 0.037. This animal was allowed free access to NaCl. This goat was maintained in nitrogen balance and was quite fat when it suddenly died of a kidney infection at the end of this period. In a more recent experiment, 6 young kids were transferred directly after weaning to a synthetic diet of similar design, except the cellulose proportion was lowered and the salt mixture contained no added silicates and the cations were fed as citrates. These kids are still growing normally at this time after consuming this synthetic diet for 2 months.

Twenty rabbits have also been fed synthetic diets of similar composition. They consume the feed readily and young animals continue to grow for about one month. In the case of the rabbits, the optimum cellulose level seems to be about 30%. Mineral mixture at a 6% level seems sufficient. Silicon does not seem essential.

Upon these purified diets young rabbits cease to grow and start losing weight in about one month. At the end of about 6 weeks these young animals develop a paralysis of the rear legs, in spite of the fact that this diet is nutritionally complete for rats. The histological picture of the degenerated muscles found in the paralyzed legs is similar to that described by Goettsch and Pappenheimer.[2] This paralysis is not due to a deficiency in the recognized B factors since it is not cured by large doses of yeast or Harris concentrate. Adult rabbits develop a similar paralysis after about 80 days. This paralysis develops upon a diet in which the protein is furnished by egg albumin as quickly as it does with casein.

In a single preliminary experiment using 7 litter mates, 5 of the young died with the paralysis while the 2 remaining were changed to a diet of alfalfa as soon as they started to lose weight. They recovered, made a normal growth and showed no muscle degeneration after being killed.

We wish to thank Dr. S. A. Asdell for assistance in the histological studies.

* Furnished as washed "Sylphrap" by the Sylvania Industrial Corp., 122 East 42nd Street, New York City.

[2] Goettsch, M., and Pappenheimer, A. M., *J. Exp. Med.*, 1931, **54**, 145.

6441

Use of Pitressin in Local Anesthesia.*

P. K. KNOEFEL.† (Introduced by C. D. Leake.)

From the Pharmacological Laboratory, University of California Medical School, San Francisco.

The use of epinephrine in solutions for local anesthesia by injection is unsatisfactory as sterilization is impossible, the solution may cause edema, and under certain conditions, may be more toxic than the local anesthetic alone.[1] However the anesthesia produced by the local anesthetic alone is often insufficient. Spagnol[2] has claimed that solutions of procaine containing a pressor substance from the posterior pituitary lobe cause prolonged anesthesia, never cause edema, may be sterilized, and are not more toxic than procaine alone. These statements are not entirely true.

Modifying the method of Rose[3] by injecting intradermally in symmetrical spots on guinea pigs 0.1 cc. of a 1% solution of procaine hydrochloride, and the same containing epinephrine or pitressin, it was found that 1 cc. of pitressin solution (20 pressor units) per 100 cc. prolonged anesthesia as much as 1-50,000 epinephrine. Half this concentration of pitressin had some effect, one-fifth none. Prolongation of local anesthesia by pitressin was not lost after sterilization in the autoclave for one hour at 15 pounds, while the epinephrine-containing solution did not differ from procaine alone after such treatment (Table I).

TABLE I.
Duration of effect of anesthetic mixtures. Ratio of mixture to procaine alone.

Concentration in 1% procaine	Unheated	Heated
Pitressin 1-500	1.0	—
" 1-200	1.7	1.5
" 1-100	4.6	—
Epinephrine 1-50,000	4.2	1.0

The toxicity of procaine on intravenous injection in rabbits is increased somewhat by the presence of 1-50,000 epinephrine. This does not occur if pitressin is present in a minimal concentration for

* Aided in part by the Christine Breon Medical Research Fund.

† Fellow of the National Research Council.

[1] Taubman, G., and Jung, G., *Arch. f. Exp. Path. u. Pharm.*, 1930, **156**, 18.

[2] Spagnol, G., *Rev. sud-americana endocrinol. immunol. quimioterap.*, 1931, **14**, 569.

[3] Rose, C. L., *J. Lab. and Clin. Med.*, 1929, **15**, 128.

prolongation of anesthesia, but if enough pitressin is added to produce a local anesthesia equal to that with procaine-epinephrine, the intravenous toxicity is increased, and to the same extent as with epinephrine (Table II).

TABLE II.

Intravenous toxicity of procaine, procaine-pitressin, and procaine-epinephrine. Mortality Ratio: No. of animals dying/No. of animals used.

mg./kg.	Procaine	Procaine+1-200 Pitressin	Procaine+1-100 Pitressin	Procaine+1-50,000 Epinephrine
20	—	—	0/3	0/2
25	0/5	—	2/5	1/3
30	1/5	0/1	2/5	3/5
35	3/5	1/3	3/4	—
40	4/5	3/3	—	—

Furthermore, pitressin does not delay absorption sufficiently to reduce the subcutaneous toxicity of a local anesthetic. Pantocain (courtesy Winthrop Chemical Co.), a local anesthetic resembling procaine, in a subcutaneous dose of 25 mg./kg. kills 6 of 8 rabbits. The same dose killed 2 of 3 rabbits although pitressin 1-100 was present, it serving only to delay the onset of symptoms. In a solution of 1-50,000 epinephrine, the same dose of pantocain produced no intoxication in 3 rabbits.

Summary. The addition of pitressin to a solution of a local anesthetic agent prolongs anesthesia on intradermal injection, and permits of sterilization. Both pitressin and epinephrine increase the intravenous toxicity of a local anesthetic. While epinephrine by delaying absorption reduces the subcutaneous toxicity, pitressin does not.

6442

Effect of Suprarenalectomy on Sugar Tolerance.

ELSIE HILL AND ALFRED E. KOEHLER.

From the Potter Metabolic Clinic, Santa Barbara Cottage Hospital.

The rabbit adapts itself well for the determination of sugar tolerance because of the convenience with which sugar can be given in the ear vein and the ease with which the blood can be withdrawn without excitement. Bilateral suprarenalectomy can readily be performed in 2 stages with good recovery of the animal but with a

variable survival period.[1] The rabbits in these experiments were all of a uniform inbred stock free of any parasitic infections. They were kept on an alfalfa-barley diet. The right gland, which was always removed at the first stage operation, lies so closely against the vena cava that its removal with its capsule intact is difficult without injury to the vessel. To overcome this difficulty, a curved clamp was applied between the gland and the vena cava including a part of the vessel. A crescent shaped portion of the vessel wall was then removed together with the gland which had previously been freed. The vena cava was then ligated by placing the ligature behind the convex portion of the clamp. This technic assured the complete removal of the right gland and caused only a slight constriction in the vena cava. The left gland was removed 7 to 10 days after the first operation.

Of 18 rabbits only 4 survived over a 60-day period; the other 14 succumbed within 20 days, with an average survival period of 14 days. The rabbits made a quick recovery from the second stage operation and the incisions were well healed without infection in a week. There was, however, a gradual weight loss for practically all the rabbits, particularly during the second week after operation, except for those rabbits which survived longer than 20 days.

To determine the sugar tolerance, 1.0 gm. of glucose per kilo was given as a 50% solution in the median ear vein of the rabbit after a 24-hour fast. The duration of injection was approximately 1 minute. In both the unilateral and bilateral suprarenalectomized rabbits the sugar tolerance was determined 7 to 10 days after operation. The average blood sugar values of 8 unilaterally operated animals (Figure 1) was practically the same before and after glucose injection as the average for normal rabbits as shown in Figure 2 and also indicated by squares in Figure 1.

The blood sugar level averages in bilaterally suprarenalectomized rabbits were practically the same before glucose injection but remained distinctly higher after glucose (Fig. 1). One bilaterally suprarenalectomized rabbit had an unusually high blood sugar 30 and 50 minutes after glucose injection which greatly increases the average value. If a weighed average is taken, the 30 and 50 minute points for the bilateral curve are materially lowered and consequently depress the slope of this curve.

These experiments indicate that the suprarenalectomized rabbit has a poorer sugar tolerance than the normal rabbit. In interpret-

[1] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1921, **57**, 135.

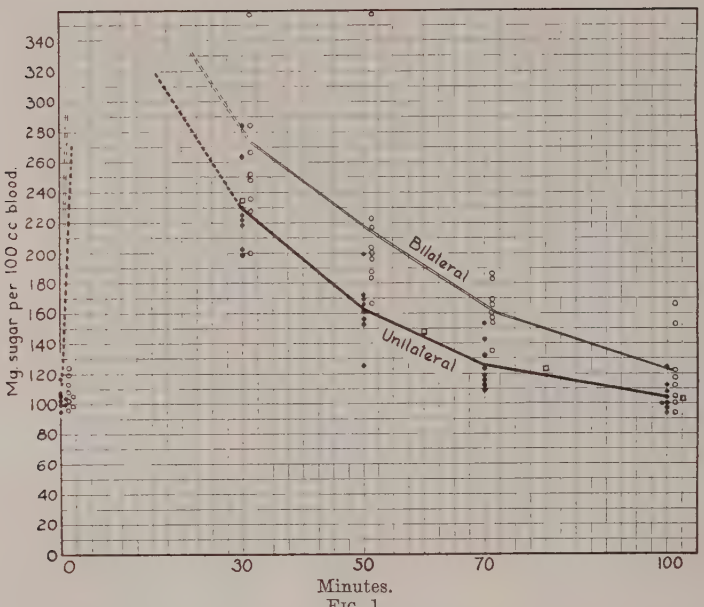

Mg sugar per 100 cc blood.
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
30
50
70
100
Minutes.
Bilateral
Unilateral

FIG. 1.

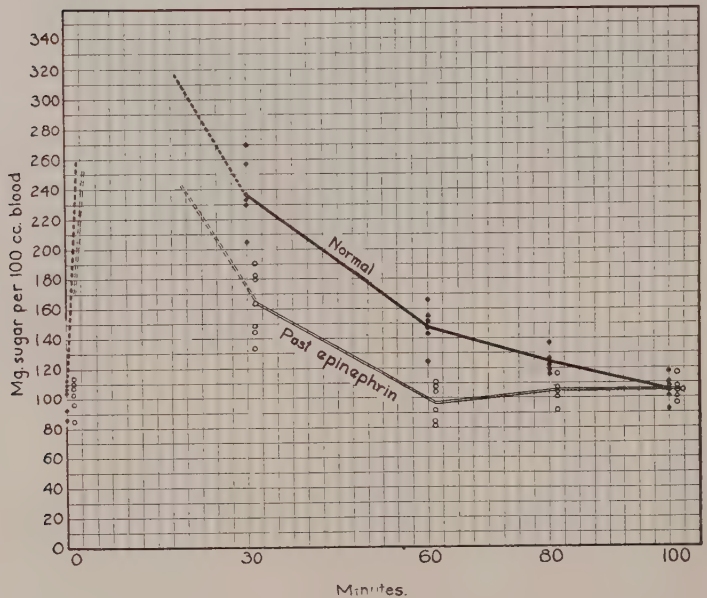

Mg. sugar per 100 cc. blood
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0
30
60
80
100
Minutes.
Normal
Post epinephrin

FIG. 2.

ing the significance of these findings, consideration must be given to (1) rate of diffusion of sugar into the tissues, (2) rate of glycogen and fat formation, (3) rate of sugar oxidation, and (4) state of nutrition previous to the test.

If epinephrin depresses sugar utilization directly, or if it depresses insulin formation, then suprarenalectomy, considered from the epinephrin standpoint alone, might well improve the sugar tolerance. If the diminished sugar tolerance after suprarenalectomy is due to decreased tissue utilization of sugar then it is possible that the suprarenal glands contain a substance other than epinephrin or possess a function favorable to carbohydrate consumption. Such a conclusion is not justified from these experiments at present because of the possibility that the decreased tolerance after suprarenalectomy may be related to the nutritional disturbances associated with diminished food intake and weight loss.

Effect of previous epinephrin administration on sugar tolerance. Sahyun and Blatherwick[2] have shown that epinephrin injection 24 hours previous to the administration of insulin greatly increases the sensitivity of rabbits to insulin. Figure 2 shows the normal fasting sugar tolerance of 6 rabbits and also 24 hours after the administration of 0.1 mg. of epinephrin per kilo. Three of the rabbits received the epinephrin daily for a week and 3 received it twice during the 12 hours preceding the 24-hour fast before the sugar tolerance. Practically no sugar was excreted in the urine (occasionally traces) after the epinephrin, so the improved sugar tolerance was not due to sugar loss. The improved tolerance and increased insulin sensitivity may be related to (1) increased insulin secretion secondary to its depression by epinephrin, (2) changes in the mobilization of carbohydrate induced by the epinephrin such as deglycogenation of muscle, and (3) increased utilization of carbohydrate or increase in its conversion to fat. Other possibilities such as the sensitization by epinephrin of nerve structures instrumental in carbohydrate metabolism must be considered.

[2] Sahyun, M., and Blatherwick, N. R., *J. Biol. Chem.*, 1928, **79**, 443.

6443

Effect of Dietary Calcium and Phosphorus on Toxicity of Lead in the Rat: Rationale of Phosphate Therapy.*

DAVID H. SHELLING.

From the Harriet Lane Home, Johns Hopkins Hospital, and the Department of Pediatrics, Johns Hopkins University.

Since the introduction of calcium salts or calcium containing foods, by Aub and his associates,[1] in the treatment of lead poisoning, numerous papers have appeared in the literature advocating such therapy. The rationale of the procedure was based on the following facts: First, the solubility of lead phosphate was found to be analogous to that of calcium phosphate; hence Aub and his associates believed that lead might be deposited in the bones in the same manner that lime salts are and thus be removed from the circulation. Second, when diets low in calcium were fed to cats, the trabeculae of the bones of the animals were diminished in size and in number as compared to those of animals fed diets to which calcium had been added. This was interpreted to mean that the addition of calcium salts to diets *in general* results in an increased storage of lime salts in the trabeculae; and since lead was supposed to behave like calcium, its deposition in the bones could be hastened or increased by furthering the process of calcification through the administration of calcium. Third, the fact that lead colic may be alleviated, almost instantly, by the intravenous administration of calcium chloride was thought to add additional evidence that calcium "drives" lead into the bones. However, the later studies of Aub and coworkers[2] discredit such an assumption since the alleviation of pain is too rapid to be due to precipitation of lead in the osseous tissue. Aub and his coworkers are, therefore, now inclined to believe that the action of calcium in this instance is to relax the intestinal musculature.

That the administration of calcium salts does not always result in improved calcification unless the phosphorus in the diet is controlled, may be inferred from the experiments of McCollum, Ship-

* Aided by a grant from Mead Johnson and Company, Evansville, Indiana.

[1] Aub, J. C., Fairhall, L. T., Minot, A. S., and Reznikoff, P., *Medicine*, 1925, **4**, 1.

[2] Fitzhugh, G., Miller, M. L., Taylor, G. W., and Aub, J. C., *Am. J. Physiol.*, 1931, **97**, 142.

ley and Park[3] on the production of rickets in rats. When animals are reared on diets in which the calcium and phosphorus ratio is about 1:1, the bones appear normal, but when this ratio is increased to about 4:1 a most florid rickets develops. Apparently the excessive intake of calcium over phosphorus results in an increased excretion of the former through the intestines as the insoluble phosphate, so that the body is robbed of lime salts which are necessary for calcification. Similarly, if the phosphorus greatly exceeds the calcium intake, the excretion of the excess of phosphorus in the feces as the insoluble calcium salt leads to rickets or, more frequently, to osteoporosis—a condition produced by Aub and associates in their experiments with cats, since their low calcium diets were relatively high in phosphorus. These derangements in calcium metabolism may be averted or rectified by adding calcium to the low-calcium diet and by either adding phosphorus to or decreasing the calcium in the low-phosphorus ration, so that the calcium and phosphorus ratio approaches a more appropriate value. Later Shipley *et al.*,[4] and others[5] demonstrated that rickets may be produced not only by diets high in calcium and low in phosphorus but also by the addition of other cations equal in molality to calcium which are excreted as insoluble phosphates in the feces. Rickets has been produced in the rat by replacing the whole or part of the calcium in ricketogenic diets with strontium, magnesium, beryllium, thallium, iron or lead. For example, when part of the calcium of the Steenbock ricketogenic diet is removed so that the calcium-phosphorus ratio approaches one, rickets is usually absent. When, however, the removed calcium is replaced by an equimolar amount of lead so that the sum of the amounts of calcium and lead is equal to the total amount of calcium in the Steenbock diet, or about 4 times that of phosphorus, severe rickets develops. The rickets may be intensified by adding calcium to the lead-containing diet, or by merely adding lead to the usual Steenbock ricketogenic diet. Obviously, aside from the toxicity of the lead, the addition of calcium to lead diets which contain an inadequate amount of phosphorus does not lead to improved deposition of either calcium or lead phosphate in the bones but, on the contrary, it inhibits such a process. The deposition of calcium phosphate in the course of normal ossifica-

[3] McCollum, E. V., Simmonds, N., Parsons, H. T., Shipley, P. G., and Park, E. A., *J. Biol. Chem.*, 1921, **45**, 333; *Bull. Johns Hopkins Hosp.*, 1921, **32**, 363.

[4] Shipley, P. G., Park, E. A., McCollum, E. V., Simmonds, N., and Kinney, E. M., *Bull. Johns Hopkins Hosp.*, 1922, **33**, 216.

[5] Park, E. A., *Physiol. Rev.*, 1923, **3**, 129.

tion, or the deposition of other insoluble phosphates such as strontium or lead, can occur only when the phosphorus intake is adequate for their deposition and for the excretion of the excess cations as the insoluble salts in the feces.

On theoretical grounds alone, it would seem logical that if the aim of therapy in lead poisoning is to deposit or excrete the lead in an insoluble and hence in an innocuous form, *i. e.*, as the phosphate, an abundance of phosphorus or foods containing phosphorus should be supplied. Certainly, the introduction of large amounts of calcium without phosphorus into the animal organisms merely diverts the available phosphorus to rid the body of the excess calcium as the phosphate and thus interferes with the formation of such a compound with lead. The correctness of such an assumption was tested experimentally in rats.

Thirty-two rats, 30-40 days old, averaging 65 gm. in weight, the offspring of healthy stock, were divided into 8 groups of 4 each. They were fed 1.5 gm. % of $2PbCO_3$-$Pb(OH)_2$ in the Steenbock-Bills stock diet[6] with and without the additions of either $CaCO_3$, Na_2HPO_4 or $3\ MgCO_3.Mg(OH)_2+3H_2O$. Four of the groups received the respective diets without vitamin D while the remainder received the corresponding diets and vitamin D in the form of viosterol 1-D in levels of 1% of the diet. The compositions of the diets are given in Table I. The animals were allowed food and water

TABLE I.
Composition of Diets Used.

Diet No. 1	Diet No. 2	Diet No. 3	Diet No. 4
Stock + 1.5 gm. $2PbCO_3.Pb(OH)_2$	Diet No. 1+1.5 gm. $CaCO_3$	Diet No. 1+0.9 gm. $3MgCO_3Mg(OH)_2+3H_2O$	Diet No. 1+2.75 gm. Na_2HPO_4 (Anhyd.)
" + Viosterol	" + Viosterol	" + Viosterol	" + Viosterol

The stock diet is that described by Bills[6] and contains approximately 0.475 gm. calcium and 0.515 gm. phosphorus %. The viosterol was diluted with olive oil to make 1D, or equal to cod liver oil in antirachitic potency. The added calcium, magnesium and phosphorus are, approximately, in equimolar amounts.

ad libitum, and their weights and changes in health and behavior were noted at frequent intervals. The effect of the variations in the calcium and phosphorus of the diets on the toxicity of lead as indicated by weight and longevity of the animals is shown in Figs. 1, 2, and 3. It is seen that among the groups *not* receiving vitamin D, the weight and longevity were the poorest in the following order:

[6] Bills, C. E., Honeywell, E. M., Wirick, A. M., and Nussmeier, M., *J. Biol. Chem.*, 1931, **90**, 619.

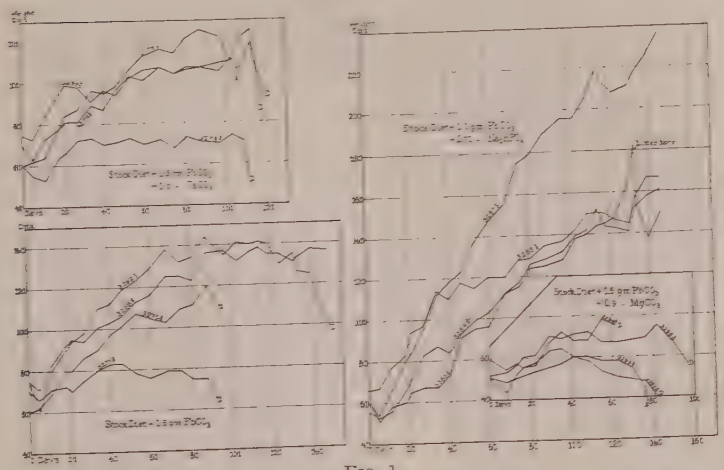


FIG. 1.

Growth curves of rats fed the stock diet and lead carbonate, with and without additions of calcium, magnesium and phosphate. The formulae for the carbonate of lead and magnesium are abbreviated in Figs. 1 and 2. The complete formulae are given in the text.

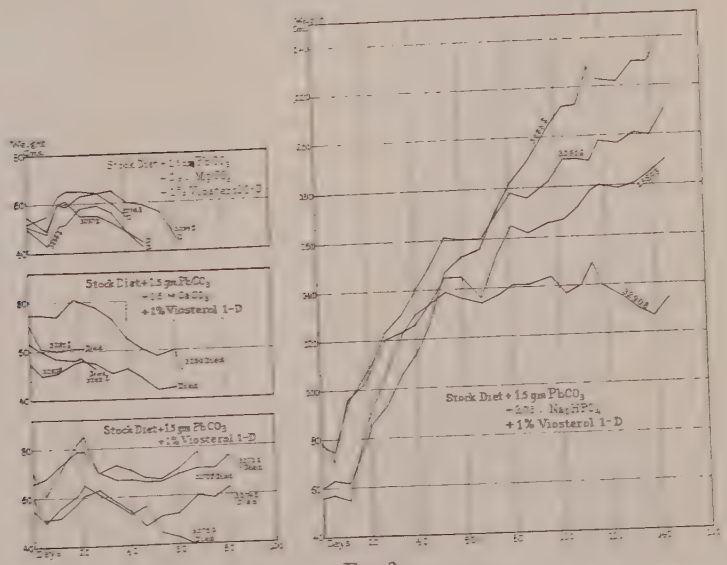


FIG. 2.

Same as Fig. 1, except that 1% of viosterol 1D was added to the corresponding diets.

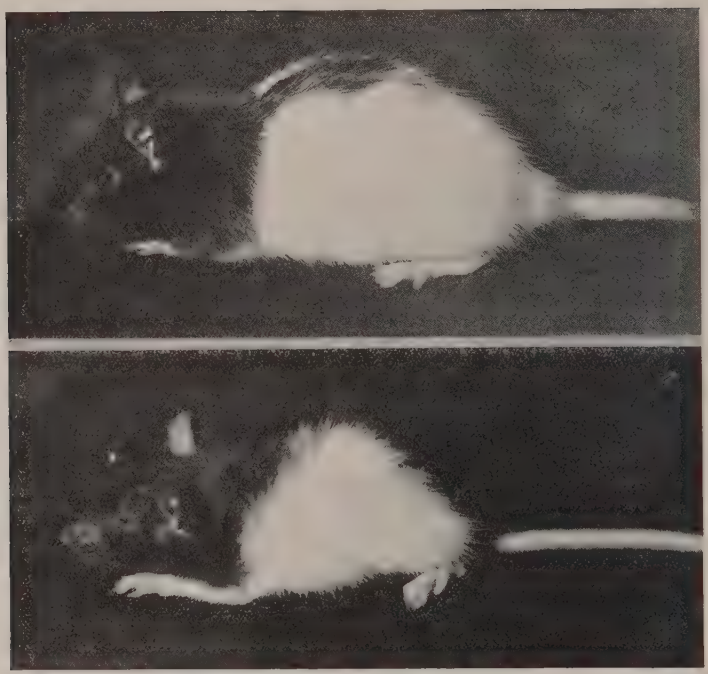


FIG. 3.
Appearance of rats poisoned with lead carbonate. *Upper:* Female, 117 days on lead and phosphate diet. Weight, 142 grams. Still living. Had litter at 133 days. *Lower:* Female, litter mate, 117 days on lead and calcium diet. Weight, 43 grams. Died on 118th day.

$MgCO_3$, > $CaCO_3$, > $PbCO_3$ (Without additions). Of the animals receiving $PbCO_3$ alone, one is still alive but is failing very rapidly. Those receiving Na_2HPO_4 gain weight steadily and appear normal outwardly. One female of this group had a litter of young after being on the diet for 133 days, but she killed her offspring soon after delivery.

With the exception of those in the Na_2HPO_4 group, which are alive and doing well, the animals receiving vitamin D all died sooner than their corresponding mates not receiving the vitamin. The interpretation of these results may be that vitamin D diverted *calcium* phosphate into the bones and allowed lead, not combined with phosphate, to circulate freely in the body fluids; whereas in the Na_2HPO_4 group the phosphate was adequate for both deposition and excretion of the cations (Ca^{++} and Pb^{++}) as the insoluble phosphate and the animals, therefore, seem well. In the latter case vita-

min D may have been helpful in depositing lead along with the calcium in the bones.

Magnesium was used in the experiments for two reasons: (1) $MgSO_4$ is frequently advocated as a remedy in lead encephalitis. (2) If phosphorus should be a determining factor in the inactivation of lead by forming an insoluble lead compound, it was anticipated that magnesium would increase the toxicity of lead because of the summation of total cations, and also because it was previously shown by Shelling, Kramer and Orent[7] and by Shipley and Holt[8] that magnesium hinders calcification, probably by increasing the solubility of insoluble phosphate compounds (Ca^{++}, Pb^{++}). The results of the experiment with magnesium are in accord with this view and indicate the danger of using soluble magnesium salts in the treatment of lead poisoning.

A few animals, not included in the above groups, were fed the stock diet and lead carbonate until they showed evidence of toxicity, as indicated by diminution of activity and loss in weight. They were then placed on the same diets to which, in one group $CaCO_3$ and in another group Na_2HPO_4, were added. Those receiving the Na_2HPO_4 improved rapidly while those getting $CaCO_3$ fared very poorly. The animals in the Na_2HPO_4 group, after being on the diet for 20 days and showing marked improvement, were suddenly changed to the high calcium diet. They lost weight very rapidly, became less active and on the 6th day one developed paralysis of the hind legs and died in convulsions during the following day. Apparently the sudden ingestion of an excess of calcium liberated the stored lead into the circulation, causing the toxic manifestations.

The application of these experimental results to the prophylaxis of lead poisoning in human beings, especially those poisoned by the gastro-intestinal tract, seems obvious. Fortunately, most diets of children and adults contain adequate amounts of phosphorus to take care of calcification and inactivation of the heavy cation lead which requires but a small amount of $PO^{=}_4$ to form an insoluble compound. In advocating calcium additions to the diets in the treatment of lead poisoning Aub and his associates advise milk, which contains, besides calcium, an abundance of phosphorus; but others, not realizing the phosphorus factor in this process, prescribe large amounts of calcium salts other than the phosphate. It would also seem that not only is phosphorus important in

[7] Shelling, D. H., Kramer, B., and Orent, E. R., *J. Biol. Chem.*, 1928, **77**, 157; *Bull. Johns Hopkins Hosp.*, 1927, **41**, 426.

[8] Shipley, P. G., and Holt, L. E., Jr., *Bull. Johns Hopkins Hosp.*, 1927, **41**, 437.

depositing lead in the bones but also in attempting to delead at a future date. This may be accomplished by giving a diet low in calcium and relatively high in phosphorus so that the cations removed from the bones are excreted as the insoluble phosphates. The metabolism data of Aub and his associates indicate that, aside from its acidity, H_3PO_4 was the most effective substance in deleading patients suffering from chronic lead poisoning.

More extensive experiments on the effect of dietary calcium and phosphorus in lead and thallium poisoning in rats, induced by oral and subcutaneous administration, are now in progress. The results of these experiments and also of those obtained with phosphate therapy in human cases of plumbism will be reported later.

6444

Relation of Pressure to Rate and Quality of Milk Secreted.*

W. E. PETERSEN AND T. V. RIGOR.

From the Division of Dairy Husbandry, University of Minnesota.

Recent work[1, 2] has shown that milk secretion takes place in the interim between milkings, and that practically all milk drawn at a milking is present in the udder at the time of milking. It is evident, therefore, that pressure must develop in the duct system as the secretion accumulates. The purpose of this work is to establish the amount of pressure developed by the accumulating secretion; the effect of such pressure upon the rate of secretion; and the maximum pressure against which milk will be secreted.

Neusch,[3] Isaachsen,[4] and Tgetgel[5] measured the pressure in the udder at milking time and reported wide differences due no doubt to a difference in distention of the gland, and as they measured the

* The data used in this paper are taken mainly from a thesis presented by T. V. Rigor in partial fulfillment for the Ph.D. degree.

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[1] Petersen, W. E., Palmer, L. S., and Eckles, C. H., *Am. J. Physiol.*, 1929, **90**, 573.

[2] Swett, W. W., *J. Dairy Sci.*, 1927, **10**, 1.

[3] Neusch, J., *Uber das Sog. Aufzieben der Milch*, 1910, Paul Parey, Berlin.

[4] Isaachsen, H., *Proc. World's Dairy Congress*, 1923, **2**, 1018.

[5] Tgetgel, B., *Schweizer Arch. f. Tierheil.*, 1926, **68**, 335, 369.

pressure from the base of the udder there is no way of knowing the variations caused by the hydrostatic pressure due to the column of milk in the udder cisterns. No reference has been found to the maximum pressure developed by not milking.

Two methods were used to measure the pressure: (1) measuring the maximum pressure developed in the udder by means of a manometer and (2) determining the maximum pressure against which milk is secreted by applying air pressure in the duct system.

To measure the pressure developed through accumulation of milk in the udder a manometer was connected to a teat cannula inserted in the teat canal. The pressure was read in centimeters height that the milk would rise from floor of the udder.

While the left gland was milked at regular intervals milk was permitted to accumulate in the right gland for 24, 36, and 120 hour intervals with a 5 day preliminary period before each trial. While the milk was drawn after these intervals the pressure was measured frequently. Two cows were used in the experiment and each trial repeated.

TABLE I.
Milk Pressure and Milk Yield.

Trial	Right Gland Delayed Milking			Left Gland Milked at Normal Intervals		
	Interval	Pressure	Milk	Interval	Pressure	Milk
Cow E 93	hr.	cm.	lb.	hr.	cm.	lb.
1	24	40	5.70	12	—	—
2	24	38	4.90	12	34	2.50
1	36	52	5.70	12	35	2.00
2	36	41	4.50	12	31	2.10
1	120	26	1.80	12	37	2.00
2	120	24	1.60	12	34	2.50
Cow 141						
1	24	50.0	3.80	12	41.0	1.80
2	24	52.2	5.70	12	45.0	2.00
1	36	50.2	3.70	12	44.0	1.80
2	36	49.2	4.70	12	41.7	2.00
1	120	37.0	2.00	12	44.0	1.60
2	120	42.0	1.50	12	45.0	1.50

Table I gives the results of the experiments. The 2 cows while developing the same maximum pressure as a result of accumulating milk in the gland required different time intervals. For cow 141 the maximum pressure of 52.2 cm. was developed at 24 hours while for cow E93 it took 36 hours. In all trials the pressure was greater at 24 hours than at 12 hours. After the maximum pressure is attained there is a fall which at 120 hours is considerably less than at 12 hours. This is due no doubt to a cessation of secretion and resorption of the products of secretion.

It is recognized that the method used above is relatively inaccurate due to the variations in hydrostatic pressure and leg pressure on the gland. It was therefore decided to ascertain the minimum air pressure required to stop milk secretion. Air pressure at a constant level was maintained for a period of 6 hours after the cow was milked and the effect upon secretion noted. Pressure was maintained from a tank into one quarter of the udder through a teat cannula. Pressure was used at various levels. Four trials were run, of which the data in Table II are typical. In all 4 trials

TABLE II.
Effect of various levels of air pressure upon the rate of milk secretion compared with normal production.

Quarter Under Pressure		Normal Quarter
Pressure mm. Hg.	Milk Yield lb.	Milk Yield lb.
10	0.9	2.8
20	0.5	2.9
25	0.1	3.0
30	0.0	2.5

the pressure of 25 mm. Hg. resulted in almost complete cessation of secretion. Pressure at 10 mm. Hg. permitted one-fourth normal secretion while at 20 mm. only about one-sixth. In one trial, pressure was maintained at 5 mm. Hg. when one-half the normal secretion was secured.

Summary. Data have been presented to show the pressures built up in the mammary gland due to accumulation of the milk secreted and the effect of such pressures on the rate of secretion. The effect of air pressure on the rate of secretion has also been reported. It is concluded that as pressure develops in the gland the rate of secretion decreases until a pressure equal to 25 mm. Hg. is developed when secretion is stopped and resorption sets in.

6445

Effect of Delayed Milking Upon Composition of Cow's Milk.*

W. E. PETERSEN AND T. V. RIGOR.

From the Division of Dairy Husbandry, University of Minnesota.

Several workers have reported upon the effect of incomplete or delayed milkings upon the fat content of the milk, but the complete picture as to what happens to milk in the udder after resorption commences has not been reported. This investigation deals with a study of the changes that take place in milk after resorption of milk begins at various intervals between milkings.

The milks used for analysis in this report were secured from the delayed milkings of the previous report, therefore representing milks retained in the udder for 24, 36, and 120 hour intervals as well as the normal 12 hour interval. The determinations made and methods used are: (1) lactose according to Bierman and Doan,[1] (2) protein according to Kjeldahl-Gunning-Arnold, (3) fat according to Mojonnier,[2] (4) total solids according to Mojonnier,[2] (5) ash according to official method, (6) phosphorus according to Briggs,[3] (7) calcium according to Kramer-Tisdall,[4] and (8) pH by potentiometer. Lactose and pH were determined on the fresh milk within one hour after drawn. In a few cases lactose was calculated by difference between total solids and sum total of the other ingredients.

The influence of delayed milkings upon the composition of the delayed milk is determined by comparison of the analysis of such milks with similar analysis of a 5-day preliminary period and with normal milkings from the left gland milked at 12-hour intervals. The experiment was repeated twice on each of 2 cows.

In Figure 1 the results are shown graphically by averaging the data secured in 2 trials on one cow, E93, which is typical. The preceding paper reveals that there is an increase in the amount of milk produced for the 24 and 36 hour periods, then a marked decrease for the 120 hour period, indicating marked resorption of the milk

* The data used in this paper are taken mainly from a thesis presented by T. V. Rigor in partial fulfillment for the Ph.D. degree.

Published with the approval of the Director as Paper No. 1111, Journal Series, Minnesota Experiment Station.

[1] Bierman, H. R., and Doan, F. J., *J. Dairy Science*, 1924, **7**, 381.

[2] Mojonnier, T., and Troy, C. H., The Technical Control of Dairy Products, Mojonnier Bros. Co., Chicago, 2nd Ed., 1925.

[3] Briggs, A. P., *J. Biol. Chem.*, 1922, **53**, 13.

[4] Kramer, B., and Tisdall, F. F., *J. Biol. Chem.*, 1921, **47**, 475.

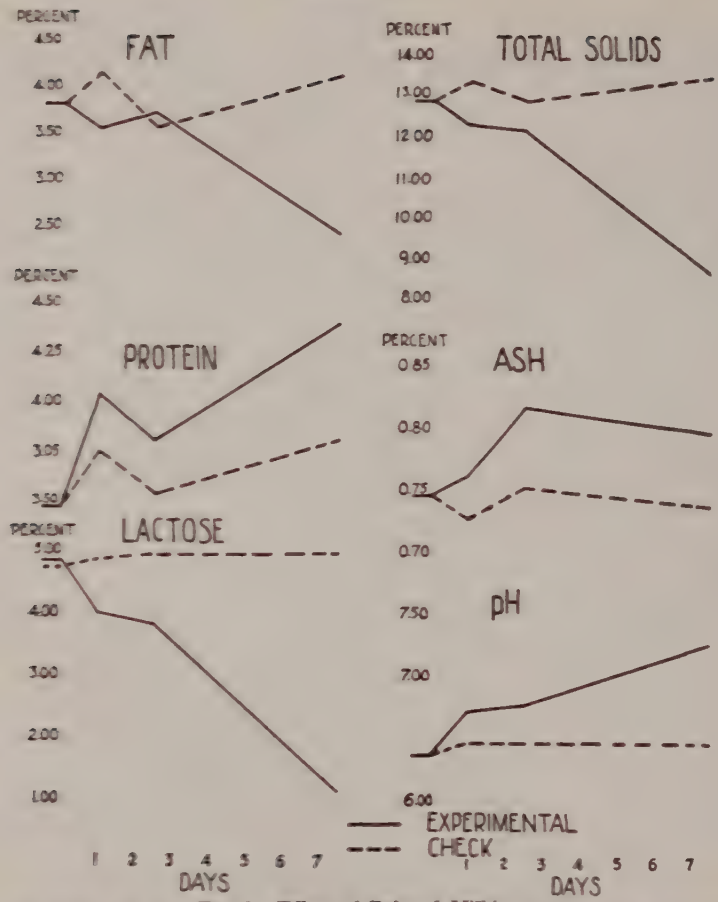

FIG. 1. Effect of Delayed Milking.

The short line on the left is the average of 2 five-day preliminary milkings. The points following represent the results of delayed milkings based upon 2 periods each of 24, 36, and 120 hour intervals.

had taken place in that time. Retention of the milk for 24 and 36 hours had little influence upon the fat content, but 120 hours caused a marked decrease in fat content.

Protein content of the milk increased with the length of period the milk was retained and the character of the protein was markedly influenced. (This phase is now being studied.) The addition of a little formaldehyde to the milk caused a solid gel to form.

The most striking effect of retention of milk in the udder is upon the lactose content. Lactose rapidly diminished from nearly a nor-

mal of 5% to less than 1% for milk retained 120 hours. Total solids diminished with retention mainly because of the decrease in fat and lactose.

Total ash increased, reaching the maximum in 36 hours. Calcium and phosphorus, however, decreased, reaching the minimum after 120 hours, which was slightly more than one-half of the normal. There was a large increase in chlorides, presumably sodium chloride. This increase in chloride content and decrease in lactose is taken as an indication that the milk is coming into equilibrium with the blood as far as the solutes are concerned.

The pH increased from 6.4 to over 7.3 for milk retained 120 hours. Just why the pH should be higher for retained milk than for the blood is problematical.

Conclusions. After milk has been permitted to accumulate in the udder to reach the maximum pressure, resorption takes place, which greatly alters the composition of the udder contents. Lactose and fats first diminish with an increase in total protein. Ash increases due to an increase in chlorides, taken to be sodium chloride, and there is a decrease in both calcium and phosphorus. The pH is increased to above that of normal blood. The character of the protein is also changed.

6446

Osmotic Pressure and Milk Secretion.*

W. E. PETERSEN AND T. V. RIGOR.

From the Division of Dairy Husbandry, University of Minnesota.

Previous work[1, 2] has shown that physical pressure exerts a marked influence upon the rate and character of milk secretion and indicates that osmotic pressure may play an important rôle in milk secretion. It is well known that milk is isotonic, but that it is not

* The data used in this paper are taken mainly from a thesis presented by T. V. Rigor in partial fulfillment for the Ph.D. degree.

Published with the approval of the Director as Paper No. 1109, Journal Series, Minnesota Experiment Station.

[1] Petersen, W. E., and Rigor, T. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 254.

[2] Petersen, W. E., and Rigor, T. V., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 257.

in equilibrium with the blood is definitely known. Davidson[3] has reported on the effect of injection into the duct system of isotonic lactose and salt solutions. No reference has been found as to the effects of hypo- and hypertonic solutions on milk secretion or as to whether or not ions exert specific influence.

This report deals with a study of the effect upon the character of the secretions of the following: 1. Isotonic solutions: lactose, saline, and Ringer's solution. 2. Hypotonic solutions: (three-fourths concentration of isotonic) lactose, saline, and Ringer's solution. 3. Hypertonic saline (1¼ concentration of isotonic). 4. Distilled water.

These various solutions were introduced into the duct system of one quarter of the udder through a teat cannula. The amount of the solution introduced was equal to the quantity of milk withdrawn. The solution was introduced immediately following a milking and left in the gland for 12 hours. The milkings were analyzed for 4 days following the injection, the same analytic technique being used as in a previous paper.[2] Comparisons are made to the secretions of a 5-day preliminary period and to the milk produced simultaneously in the corresponding normal quarter.

Figure 1 presents graphically the effect of isotonic saline solution. Protein, ash, and pH are significantly increased with a decrease in lactose. Changes in fat and total solids are not significant. Milk yield dropped to less than one-third normal for the first milking following injection of the saline solution. All constituents as well as yield returned to normal in 2 to 4 days. Calcium and phosphorus contents were unaffected.

Figure 2 shows the effect of Ringer's solution. A marked depression of fat, lactose, and total solids is noted, reaching the minimum for the first milking following injection and becoming normal in 4 days. Protein and ash were markedly increased, reaching the maximum in second day after injection and becoming normal in 4 days. Yield of milk was reduced to less than a tenth normal and both calcium and phosphorus increased significantly. All returned to normal in 4 days.

Figure 3. With the exception of fat, isotonic lactose had the same effect upon the character of secretion as did the saline solution. The yield was not depressed to the extent it was with Ringer's and recovery to normal was effected in less than half the time.

The effect of hypotonic solutions is shown in Figures 4, 5, and 6. The variations on fat content are not significant. Protein increases

[3] Davidson, F. A., *J. Agr. Research*, 1926, **33**, 873.

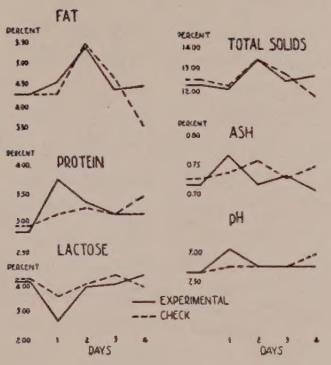

FIG. 1

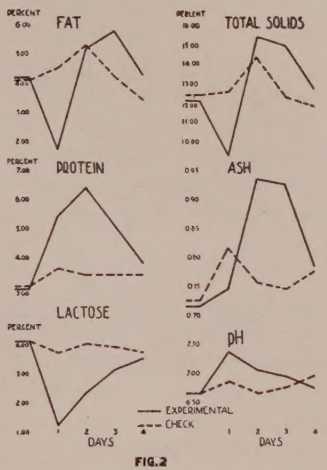

FIG. 2

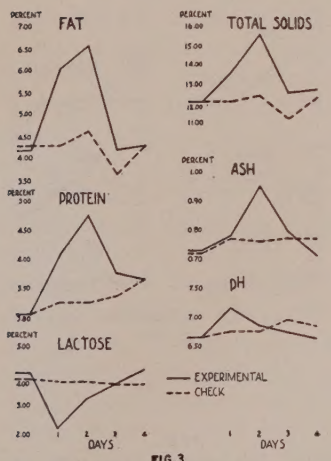

FIG. 3

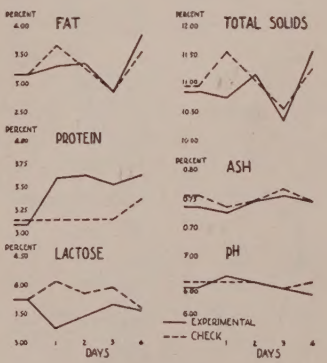

FIG. 4

FIG. 1. *Effect of isotonic saline solution upon milk secretion.* The short line on the left is the average of a 4-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

FIG. 2. *Effect of Ringer's solution upon milk secretion.* The short line on the left is the average of a 5-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

FIG. 3. *Effect of isotonic lactose solution upon milk secretion.* The short line on the left is the average of a 5-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

FIG. 4. *Effect of hypotonic saline solution upon milk secretion.* The short line on the left is the average of a 4-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

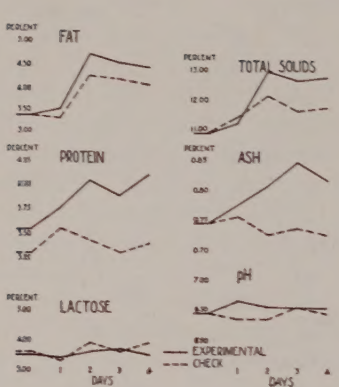

FIG. 5

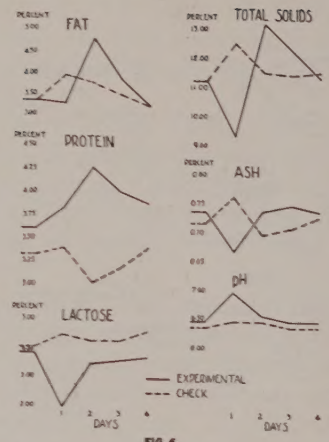

FIG. 6

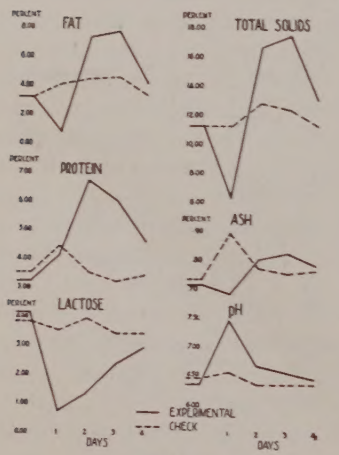

FIG. 7

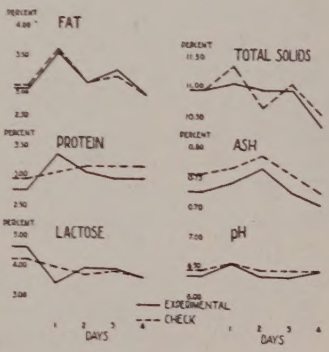

FIG. 8

FIG. 5. *Effect of hypotonic Ringer's solution upon milk secretion.* The short line on the left is the average of a 4-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

FIG. 6. *Effect of hypotonic lactose solution upon milk secretion.* The short line on the left is the average of a 4-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

FIG. 7. *Effect of hypertonic saline solution upon milk secretion.* The short line on the left is the average of a 5-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

FIG. 8. *Effect of distilled water upon milk secretion.* The short line on the left is the average of a 4-day preliminary period. The points following are for the subsequent milkings expressed in daily averages.

and does not return to normal in 4 days following the injection. Lactose slightly depressed except for "hypotonic" Ringer's solution where it is unaffected. Ash is increased for Ringer's and unaffected for the others. The pH increased for all 3. The hypotonic solutions depressed the milk yield to a much less extent than the isotonic solutions. In order of depression they were lactose most, Ringer's and saline least, although differences are not significant. The calcium and phosphorus content was unaffected except for the lactose injection when they were about three-fourths normal for the first milking following injection.

Effects upon the composition of milk following the injection of hypertonic saline solution (1¼ normal) are given in Figure 7. The effects are in the same direction only much more marked, except for ash, which increased in the milk from the corresponding normal quarter. Protein more than doubled while lactose dropped to one-fifth normal. The yield dropped from a normal of 2,000 cc. to less than 100 cc. and returned to less than one-half normal after 4 days. Both calcium and phosphorus dropped to about one-third normal for the first day following injection and normal on the second day. The milk appeared very abnormal, being watery with the proteins precipitating out immediately after milking.

The effect of distilled water is shown in Figure 8. Comparatively minor effects are to be noted for lactose (slightly depressed) and protein (slightly increased) other ingredients including calcium and phosphorus are not significantly effected. Yield dropped about 20% on first milking following injection and returned to normal on the second day.

Conclusions. From the above it becomes evident that the concentration of solutes affects both the character and amount of secretion. The depressing effect upon the amount of secretion is in direct proportion to the concentration of solutes. The amount of secretion produced following hypertonic solution is about 1/20 normal; isotonic about one-fifth normal; hypotonic one-half normal, and water four-fifths normal. This clearly indicates a selective absorption of the solutes and not a simple equilibrium phenomenon through a semi-permeable membrane. If the latter were true, water should pass into the gland from the blood and increase the amount of secretion.

As there is no significant difference between the effects of saline solution, Ringer's solution, and lactose it may be concluded that electrolytes exert no greater effect than non-electrolytes or a more balanced mixture of electrolytes such as Ringer's solution.

That the effect of an injection lasted for more than one day indicates a disturbance in secreting cell, requiring a little time to overcome. It is significant that protein in the secretion increased in the order of increase of solutes in the injected solution which is reverse to the amount of secretion.

The picture of the character of the secretion following an injections of solution approximates that of milk retained in the udder, indicating that normal milk secretion depends upon a complex selective equilibrium.